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BIOLOGICAL EFFECTS OF SHORT,  
HIGH-LEVEL EXPOSURE TO GASES: AMMONIA

PHASE REPORT

PREPARED BY

Llewellyn Legters, M.D.

May 1980

SUPPORTED BY

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND  
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-79-C-9086

Enviro Control, Inc.  
One Central Plaza  
11300 Rockville Pike  
Rockville, Maryland 20852

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FEB 4 1981  
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Project Officer:  
Mary C. Henry, Ph.D.  
Environmental Protection Research Division  
U.S. Army Medical Bioengineering Research and Development Laboratory  
Fort Detrick, Frederick, Maryland 21701

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) This report presents an analysis and synthesis of the available literature concerned with possible health and performance effects of exposures to ammonia. The US Army's concern is with short, high-level exposures that may exceed present threshold limit values of the American Conference of Governmental Industrial Hygienists (25 ppm or 17 mg/m <sup>3</sup> as a TWA and a ceiling of 35 ppm or 24 mg/m <sup>3</sup> for 15 minutes). The organs primarily affected by exposure to ammonia gas are the respiratory tract		

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and the eyes. During brief exposures to concentrations of 500 ppm (348 mg/m<sup>3</sup>) or less, the biologic responses are immediate, reversible, and mainly irritant. Below 50 ppm (35 mg/m<sup>3</sup>), there are no significant effects, except that the odor of ammonia is detectable. Between 50-100 ppm (35-70 mg/m<sup>3</sup>), most people experience some degree of irritation of the eyes, nose and throat. There is some evidence indicating that personnel may become acclimated to the irritant effects after only 1 or 2 weeks of intermittent exposure at these levels. Around 130 ppm (90 mg/m<sup>3</sup>), approximately half of exposed personnel experience lacrimation. Lacrimation has been reported in subjects inhaling 500 ppm (348 mg/m<sup>3</sup>) of ammonia via a face mask, which should have prevented direct contact of ammonia with the eyes. This suggests that wearing protective eye goggles may not completely prevent lacrimation. There is great variability in the levels of exposure found to be tolerable by uninured subjects, ranging from 110 ppm (76 mg/m<sup>3</sup>) for 2 hours (some of the same subjects found 140 ppm or 97 mg/m<sup>3</sup> to be intolerable after about 1 hour of exposure) to 500 ppm (348 mg/m<sup>3</sup>) for 30 minutes in the different studies reviewed. Temporary aberrations in respiratory physiology, manifested either by increased or decreased ventilation minute volume, have been reported at concentrations over 150 ppm (104 mg/m<sup>3</sup>). Based on very limited evidence, the least amount producing coughing would appear to be somewhere between 500-1000 ppm (348-695 mg/m<sup>3</sup>). Data on possible delayed, reversible or irreversible effects from repeated, short, high-level exposures is contained exclusively in animal studies. Extrapolation of the animal data to man is complicated by the species. Pigs and rats appear to be the most susceptible of the several species used. At concentration-time (Ct) doses around theoretical maximums under military operational conditions, pigs and rats have exhibited tracheal mucosal damage; however, similar findings were not reported in other studies using the same species and similar or higher dosage (Ct) values. As with the immediate irritant effects, concentration, rather than duration or exposure or Ct values, would appear to be the most significant determinant of effects under conditions of prolonged (continuous or repeated) exposure. Based on consideration of all data presented in the available literature, it is concluded that anticipated exposures of military personnel, even though repeated, will not produce any permanent health effects. Expected effects will be immediate, reversible, in the category of harassing, and significant only to the extent that they may affect operational efficiency to some degree.

## EXECUTIVE SUMMARY

The overall purpose of this project is to characterize the biological responses of short, high-level exposures to four gases associated with certain Army weapons systems (ammonia, carbon monoxide, sulfur dioxide and the nitrogen oxides). This report analyzes and synthesizes the available literature concerned with possible health and performance effects of exposure to ammonia.

Ammonia is generated by the combustion of ammunition propellants, especially those formulations containing nitroguanidine. Exposure of soldiers to combustion emissions may occur during training with the various weapons systems or during combat. Armored vehicle crewmen may be especially vulnerable because of the closely confined and sometimes poorly ventilated space inside the vehicles and because of the proximity of personnel to the emission sources. Exposures are expected to be intense (above threshold limit values recommended by the American Conference of Governmental Industrial Hygienists), brief (1 hour or less) and repeated (1 to 6 times daily for periods of 1 to 14 days).

Threshold limit values for use in the workplace would appear to have limited application in the military setting; the basis for their selection is the protection of workers against nose and throat irritation and the minimization of complaints of discomfort among office workers and similarly uninured individuals. Selection of maximum allowable concentrations for use in the military, more appropriately it would seem, should be based on considerations of casualty prevention (i.e., immediate incapacitation or delayed health effects) with a view toward minimizing effects that would impair operational efficiency. To this end, an effort has been made to identify threshold levels at which effects may be expected to occur, or applicable concentration-time relationships; the nature and extent of possible effects when such levels are exceeded; and gaps and inconsistencies in available data, which is the basis for identifying areas where follow-up research may be required.

The data on which this report is based were derived by the collection, critical review and evaluation of published and unpublished literature and research reports. The main sources of information were the various computer data bases, especially MEDLINE and its back files, TOXLINE, TOXBACK, NTIS AND NIOSHTIC. The data of greatest interest are contained in a very few studies of short, high-level exposures in man. These reports deal mainly with the immediate, reversible, irritant effects. Except for one report on humans, data on possible effects of prolonged exposure, either in continuous or repeated doses, are contained in reports of animal studies. Reports of accidental human exposure have also been reviewed. While the exposure levels in such instances were probably well above any likely to be encountered in the military environment, the reports are instructive with respect to the nature and extent of the pathology produced by acute ammonia gas inhalation at very high concentrations.

The organs primarily affected by exposure to ammonia gas are the respiratory tract and the eyes. The irritant effects are immediate, with

onset as soon as exposure begins, primarily concentration-dependent, and, except possibly under conditions of prolonged exposure, probably completely reversible at concentrations of 500 ppm (348 mg/m<sup>3</sup>) and below.

Below 50 ppm (35 mg/m<sup>3</sup>), there are no significant effects, except that the odor of ammonia may be detectable. Between concentrations of 50-100 ppm (35-70 mg/m<sup>3</sup>), most personnel will experience some moderate degree of irritation of the eyes, nose and throat; however, the degree of discomfort should not be so severe as to interfere with mission accomplishment. Personnel apparently may become acclimated to the irritant effects of the gas after only 1 or 2 weeks of intermittent exposure at these levels. The adaptive response appears to be incomplete, so that excursions above 150 ppm (104 mg/m<sup>3</sup>) will produce signs of irritation in personnel previously acclimated at lower concentrations.

Perhaps the most significant irritant effect from the military standpoint is the lacrimation that will occur in approximately 50 percent of personnel exposed to concentrations around 130 ppm (90 mg/m<sup>3</sup>). Lacrimation may be expected to impair operational efficiency because of interference with task involving visual discrimination (e.g., reading instruments and maps and gun-sighting). Data from one study where lacrimation was produced, even though ammonia was inhaled through a face mask and should not have come into direct contact with the eyes, suggest that providing troops with protective eye goggles may not completely prevent lacrimation. Because of its potential operational significance, this point perhaps deserves further study. It would also be useful to know if repeated, short exposures to lower concentrations attenuate the lacrimatory effect during subsequent exposures to higher concentrations.

There is great variability in concentrations reported to be tolerable by uninured subjects. In one study, all subjects tolerated exposure to 100 ppm (76 mg/m<sup>3</sup>) for 2 hours (some of the same subjects, however, found 140 ppm or 97 mg/m<sup>3</sup> to be intolerable after about 1 hour of exposure), whereas in another experiment, subjects tolerated exposure to 500 ppm (348 mg/m<sup>3</sup>) for a half hour. This variability between subjects in the different studies is presumed to be due to differences in motivations.

Exposure to concentrations between 150-500 ppm (104-348 mg/m<sup>3</sup>) produced temporary aberrations in respiratory physiology, manifested in one study by increased ventilation minute volume (tidal volume and respiration rate both increased) and in a second study by decreased ventilation minute volume (due either to decreased respiration rate or tidal volume, depending on concentration). In the first case, subjects were at rest, and in the second, subjects were undergoing submaximal exercise, which may account for the discrepancies in the findings. One might presume that during concurrent exposures, greater respiratory volumes would tend to hasten the onset of effects of the other toxicants (e.g., carbon monoxide).

On theoretical grounds the threshold level of ammonia that produced coughing may be considered as the level at which the capacity of the upper respiratory tract to adsorb the gas has been exceeded, resulting in penetration

of (and possible damage to) the lower tract. In the older literature, the threshold cited as producing coughing is 1720 ppm (1195 mg/m<sup>3</sup>); however, more recent data suggest that the threshold is greater than 500 ppm (348 mg/m<sup>3</sup>), but less than 1000 ppm (695 mg/m<sup>3</sup>).

Only one human study addresses the question of possible adverse health effects produced by repeated exposures. Subjects exposed to varying concentrations (25-100 ppm; 17-70 mg/m<sup>3</sup>) of ammonia for varying periods (2-6 hours) daily, 5 days per week, for 6 weeks, showed decreasing signs of irritation of the mucous membranes of the eyes, nose and throat over the 6 week observation period, and no evidence of adverse health effects. Evidence of possible delayed, reversible or irreversible effects of repeated exposures is contained in animal studies, and extrapolation of the data from animals to man is complicated by the apparent wide range of susceptibilities among the species.

The theoretical worst-case concentration-time (Ct) dosage value under military operational conditions is estimated to be around 35,000 ppm-hours. This estimate is based on the highest peak concentration of ammonia found recorded in US Army reports of tests in armored vehicles (410 ppm; 285 mg/m<sup>3</sup>) and on expectations with respect to possible maximum durations and frequencies of exposure (410 ppm for 1 hour, 6 times per day, for 14 days). Tracheal mucosal damage has been demonstrated in pigs exposed to 100 ppm (70 mg/m<sup>3</sup>) of ammonia continuously for 2 weeks (CT = 33,600 ppm-hours) and in rats exposed to 100 ppm (70 mg/m<sup>3</sup>) of ammonia repeatedly, 5 hours per day, 5 days per week, for 12 weeks (Ct = 30,600 ppm-hours). Pigs and rats appear to be among the most susceptible of the several species used.

Excluding possible differences in species susceptibility, failure by other investigators to corroborate these findings in any of several species (in some cases at even higher Ct values) may be due to differences between studies with respect to exposure schedules (e.g., there is some evidence to suggest that intermittent exposures may be less toxic than continuous exposures) or endpoint determinations (e.g., different investigators may have examined the target organ tissues in greater or lesser detail than others).

Definitive answers to questions about possible reversible or irreversible effects in man due to repeated, short exposures to high levels of ammonia will require additional research. While the animal data created some slight concern about the possibility of tracheal mucosal damage under conditions of repeated, worst-case exposures, it is concluded that such effects are improbable. Rather, expected effects will be immediate, reversible, in the category of harassing, and significant only to the extent that they may affect operational efficiency to some degree.

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## I. INTRODUCTION AND BACKGROUND

The overall problem addressed by this project is the potential exposure of soldiers to carbon monoxide (from engine exhaust, auxiliary generators and explosives), ammonia (from explosives, especially those formulations containing nitroguanidine), oxides of nitrogen (from explosives), and oxides of sulfur (from explosives and engine exhaust). The exposures are likely to be intense (above present threshold limit values of the American Conference of Industrial Hygienists), brief (1 hour or less), and repeated (1 to 6 times daily for periods of 1 to 14 days). Such exposures may occur during the training of soldiers with the various weapons systems or during actual combat.

In this connection, it seems appropriate to note that threshold limit values (TLV) developed for use in the workplace would appear to have limited application in the various military situations under consideration. The present TLV for ammonia (25 ppm\* as a TWA and a short-term exposure limit of 35 ppm for 15 minutes)<sup>31</sup> was selected to protect workers against nose and throat irritation and to minimize complaints of discomfort among office workers and similar uninjured individuals. It would seem more appropriate to base selection of maximum allowable concentrations for use in the military upon considerations of casualty prevention (i.e., immediate incapacitation or delayed health effects) with a view toward minimizing effects that will reduce operational efficiency.

This report presents an analysis and synthesis of the available literature concerned with possible health and performance effects of exposure to ammonia; specifically, the possible immediate and reversible effects, mainly irritant, of single, short, high-level exposures and the delayed, reversible or irreversible effects of repeated exposures. An effort has been made to identify threshold levels at which effects may be expected to occur, or applicable concentration-time relationships; the nature and extent of effects when such levels are exceeded; and gaps and inconsistencies in available data, which is the basis for identifying areas where follow-on research may be required.

While exposures to emissions from ammunition propellants, including ammonia, may be encountered by soldiers in a variety of operational settings, the US Army's concern about the potentially deleterious effects of

\*Throughout the remainder of the report, concentrations are expressed as parts per million (ppm). To convert ppm to mg/m<sup>3</sup>, multiply ppm by 0.695. This constant is derived by dividing the molecular weight of ammonia (17 g) by the volume 1 mole of gas would occupy at 25°C (298°K) and 1 atmosphere of pressure, multiplied by 10<sup>3</sup>/m<sup>3</sup> (to convert to m<sup>3</sup> units).

various air pollutants previously has focused on exposures in various armored vehicles. This focus of attention seems warranted, as armored crewmen may be especially vulnerable to the adverse effects of exposure to the toxicants in question because of the closely confined and sometimes poorly ventilated space inside the vehicles and because of the proximity of personnel to the emission sources.

One of the first attempts by the Army to resolve the problem of exposures to engine exhaust and ammunition propellant emissions dates back to 1943.<sup>26,27</sup> The stated purpose of the 1943 reports was "...to determine the extent of the hazard from fumes released by firing of the weapons in the M4A4E1 tank with 105 mm howitzer and in the M7 tank." The sources of toxic gas production and accumulation considered were the weapons systems, especially with respect to their construction and placement within the tank. The reports provide insights into the overall nature and potential severity of the problem of toxic gases in armored vehicles. The main findings of the two studies were:

- In general, the atmospheric conditions inside the tanks were completely unsatisfactory.
- The blood of crew members showed dangerous levels of carboxyhemoglobin (COHb).
- Sufficient ammonia was present to cause considerable eye and nose irritation.
- The greater the workload of a crew member the greater the respiratory rate and the level of COHb in the blood.
- The turret machine gun proved especially dangerous. In one study, the blood COHb increased at an alarming rate to 23 percent in only 9 minutes, and the concentrations of carbon monoxide and ammonia proved so intense that operation of the weapon was considered extremely difficult and unreliable. In the second study, the testing had to be discontinued early because of the immediate danger to the crew. Further, the ammonia levels were so extreme that turret crew members were unable to execute their normal tasks effectively.
- The unsatisfactory performance of certain crew members was due to the trapping of these gases by poor gun placement and mounting and inadequate ventilation.

In 1954, the US Army Human Engineering Laboratory, Aberdeen Proving Ground, Maryland, issued another report, "The Relation of Toxic Gases to Equipment Design,"<sup>28</sup> in which the concentrations of ammonia measured during weapons firing in armored vehicles ranged from 105-410 ppm. More recently, concentrations of ammonia recorded in the Main Battle Tank (XM-1) during development tests at Aberdeen Proving Ground seldom exceeded 100 ppm.

## II. APPROACH TO THE PROBLEM

The approach to the work involved the following major tasks:

- The identification of information sources
- The preliminary screening of information before acquisition
- The assessment of the availability of sufficient literature to perform remaining work elements
- The acquisition of the literature
- The critical review of documents for scientific validity
- The evaluation of biologic response data in terms of behavioral, performance and health effects, both immediate and delayed, and reversible and irreversible.

The main sources of information were the various computer data bases, especially MEDLINE and its backfiles, TOXLINE, TOXBACK, NTIS and NIOSHTIC. The computer search was performed by first selecting key terms describing substance and exposure (see Figure B-1 of Appendix B). Only articles containing one or more terms from each of three sets of key words were retrieved. Due to the paucity of relevant articles produced by this first attempt, the search method was revised. The second search eliminated all exposure-level limiters and included only terms and synonyms for substance and biological effects (see Figure B-2 of Appendix B). Again, only articles containing one or more terms from both sets were retrieved by the computer.

All materials yielded by the search of the data bases were screened by the Principal Investigator to identify articles apparently relevant to the study. The screening was based on the content of article abstracts (when available) and on the presence of keywords in article titles. Full-text copies of all apparently relevant articles were then secured for critical review and evaluation. In addition to confirming the relevance of the material to the present study, the critical review involved determinations of the adequacy and appropriateness of the experimental design, the accuracy and validity of the statistical analyses performed, and the correctness of the conclusions in light of the data analysis.

Upon completion of the search of the data bases and the screening of materials yielded by the search, it was apparent there were very few documents on short, high-level exposures to ammonia in either animals or man. Review of the references listed in key articles and other basic sources confirmed that the search had identified nearly all documents of any potential value to the project. The relative paucity of data on ammonia, as well as the absence of reports of serious adverse effects under anticipated conditions of exposure, resulted in an early decision to terminate further searching and to prepare a report based on review and analysis of the

literature already collected. This does not mean there are significant omissions of articles bearing directly on the problem of short, high-level exposures to ammonia (as far as can be ascertained, all such key articles have been included) but rather that not all of the available literature of possible peripheral interest has been collected and reviewed (e.g., reports of accidental human exposures to unmeasured concentrations, probably in the range of several thousands parts per million).

The report is divided into the following sections:

- Summary of Effects and Conclusions, which presents the main findings of the literature review in summary fashion and identifies significant gaps and inconsistencies.
- Discussion of the data presented in the literature, leading to the main findings, gaps and inconsistencies.
- Suggested Follow-on Work, in which possible additional research is proposed to fill in major gaps in the information or to resolve discrepancies.
- Literature Review and Analysis (attached as Appendix A), which is a presentation of the purpose, methods and findings of each key article, followed by a critical analysis, as appropriate, of the experimental design, statistical methods, and the correctness of the conclusions.

### III. SUMMARY OF EFFECTS AND CONCLUSIONS

In presenting the summary of effects data derived from the various studies reviewed, it is perhaps appropriate once again to define the limits of possible ammonia gas exposures to which soldiers may be subjected. It will be recalled that the US Army's concern is with exposures exceeding present threshold limit values of the American Conference of Governmental Industrial Hygienists for durations of 1 hour or less, 1 to 6 times daily, for periods of 1 to 14 days. Actual peak concentrations of exposure may be in the range of several hundred parts per million. Such levels may be encountered in the confined and sometimes poorly ventilated space of various armored vehicles.\*

The implied ultimate purposes of this report are: (1) to attempt to define "safe" exposure limits, meaning levels below which immediate or delayed health or performance effects need not be anticipated; (2) to ascertain the nature and extent of health and performance effects if such levels are exceeded; and (3) to identify gaps in the information presently available, which will suggest areas where additional research is required.

To this end, the report has focused on the very few studies of short, high-level exposures in man,<sup>1-6</sup> which have dealt primarily with the immediate irritant effects of the gas. Except for one report of repeated exposures over a period of several weeks,<sup>4</sup> exposures were in the range of 30-500 ppm for periods up to 30 minutes. At the opposite extreme in terms of dose are the reports of accidental human exposures,<sup>19-24</sup> which are concerned primarily with clinical descriptions and postmortem findings in patients exposed to very high (unmeasured) concentrations, and a few animal studies<sup>12,18</sup> at concentrations in the lethal range (around 10,000 ppm). Between these extremes, data are contained exclusively in animal studies. These are either highly specialized studies dealing with the effect of short, high-level exposures (e.g., 45-minute exposures to concentrations up to 3000 ppm) on tracheal ciliary activity and the adsorption of ammonia in the upper respiratory tract of rabbits and rats<sup>13-17</sup> or studies of the histopathologic effects of long, high-level exposures (52-1100 ppm, administered either in repeated or continuous doses, over periods as long as 18 weeks).<sup>7-11</sup> Extrapolation of the animal data to man is complicated by the apparent wide range of susceptibilities to ammonia among the several species.

\*Peak concentrations of ammonia as high as 410 ppm have been recorded;<sup>28</sup> however, generally, concentrations in armored vehicles during weapons firing have seldom exceeded 100 ppm.<sup>29</sup>

#### A. Immediate, Reversible, Irritant Effects

Levels at which the various irritant effects of ammonia have been demonstrated in man are summarized in Table 1. At these levels, the organs primarily affected are the eyes and the upper respiratory tract. The irritant effects are immediate, with onset as soon as exposure begins, are primarily concentration-dependent, and, except possibly under conditions of prolonged exposure, are probably completely reversible at concentrations of 500 ppm and below.

Below 50 ppm, there will be no significant effects, except that the odor of ammonia may be detectable.<sup>5,33</sup> Between 50 and 100 ppm, most people will probably experience some moderate degree of irritation of the eyes, nose and throat; however, the degree of discomfort should not be so severe as to interfere in any way with mission accomplishment, and concentrations at this level should be able to be tolerated by all personnel.<sup>3,5,6</sup>

Subjects apparently may become acclimated to the irritant effects of ammonia after only 1 or 2 weeks of intermittent exposure to varying concentrations for varying durations; however, the only available data on humans come from exposures in the range of 25-100 ppm. At these levels, the adaptive response apparently is incomplete, so while signs of irritation diminished over a 6-week schedule of intermittent exposures at the 25-100 ppm level, subjects experienced lacrimation during excursions above 150 ppm.<sup>4</sup> The data are insufficient to determine the optimum exposure schedule to prevent or reduce effects resulting from subsequent challenges to higher levels or to determine whether one might expect soldiers to become adapted under anticipated operational conditions.

Around 130 ppm, approximately half of exposed personnel will experience lacrimation,<sup>6</sup> which may be expected to impair operational efficiency because of interference with tasks involving visual discrimination (e.g., reading instruments and maps, and gun-sighting). More precise data are needed on the least amounts producing lacrimation among the most susceptible individuals. The data from the study by Silverman et al.<sup>1</sup> in which lacrimation was produced even though the ammonia was inhaled through a face mask and should not have come into direct contact with the eyes, suggest that providing troops with protective eye goggles may not completely prevent lacrimation. Because of its potential operational significance, this point perhaps deserves further careful study. It would also be useful to know in the same connection if repeated, short exposures to lower concentrations (say, in the range of 100 ppm) could be expected to produce attenuation of the lacrimatory effect during subsequent exposures to higher concentrations (say, in the range of 110-150 ppm). The data from the study by Ferguson et al.<sup>4</sup> suggest that acclimation at lower levels (in the range of 25-100 ppm) may not prevent lacrimation during excursions above 150 ppm.

TABLE 1

Threshold Levels and Ranges for Significant Immediate,  
Reversible (Mainly Irritant) Effects in Man

Threshold Level or Range (ppm)	Effects	Reference
20-30	Odor easily noticeable	5,33
50-72	Produces moderate irritation of the eyes, nose and throat in most subjects	3,5,6
110	Tolerated by all uninured subjects for 2 hours	3
134	Produces lacrimation in 50% of uninured subjects	6
140	Tolerated by all uninured subjects for 0.5 hours; only by highly motivated subjects for 2 hours	3
150	Produces lacrimation in subjects previously acclimated at 25-100 ppm for varying durations	4
150-500	Produces changes in ventilation minute and tidal volume and respiration rate	1,2
1000	Produces coughing	1



From the military standpoint, the range of concentrations that personnel can (or will) tolerate would appear to be of considerable importance. In the several human studies, there was great variability in the levels found to be tolerable by uninured subjects. Values ranged from 110 ppm for 2 hours (a level tolerated by all subjects in the study by Verberk)<sup>6</sup> to 500 ppm for 30 minutes (a level tolerated by Silverman's six subjects inhaling ammonia through a face mask).<sup>1</sup> Some of the so-called nonexpert subjects in Verberk's study felt compelled to escape the contaminated environment during exposures to 140 ppm.<sup>6</sup>

One suspects that the main difference accounting for the widely varying tolerance levels of subjects is the degree of their motivation or determination. To the extent that soldiers generally will be highly motivated, most especially if engaged in combat where survival may depend on mission performance, it seems reasonable to expect that soldiers' tolerance levels will be among the highest. It follows, however, that at some concentration above 500 ppm, all exposed individuals (no matter how well motivated) will seek escape from the contaminated environment.

While there is no evidence that single exposures up to 500 ppm for 30 minutes will produce any irreversible health effects,<sup>1</sup> brief (20-30 minutes) exposures to concentrations of 150 ppm and above did produce temporary aberrations in respiratory physiology, manifested by either increased ventilation minute volumes (because of increased tidal volume and respiration rate)<sup>1</sup> or decreased ventilation minute volumes associated with either increased tidal volume and decreased respiration rate at 151 ppm, or decreased tidal volume and increased respiration rate at 205 and 335 ppm.<sup>2</sup> The discrepancies in the findings of the two groups of investigators may be due to differences in the level of physical activity of subjects. The findings may be of no practical significance; however, one might presume that during concurrent exposures, greater respiratory volumes would tend to hasten the onset of effects of the other toxicants (e.g., carbon monoxide).

The threshold level that will produce coughing may be of some importance, as coughing may signify that the adsorptive capacity of the upper respiratory tract has been exceeded and that significant concentrations of the gas are penetrating into the lower passages. Based on work in rats and rabbits by Cralley<sup>13</sup> and Dalhamn and colleagues,<sup>14-17</sup> it would appear that at concentrations above the threshold, one might expect damage of the mucosa of the lower respiratory passages to occur. The least amount that causes coughing in man is cited in Henderson and Haggard as 1720 ppm;<sup>32</sup> however, the data presented by Silverman *et al.*<sup>1</sup> suggest that the threshold may be considerably lower. The latter investigators were unable to do their experiment at 1000 ppm because of coughing by their subjects. Coughing was not induced at 500 ppm.<sup>1</sup>

#### B. Possible Delayed, Reversible or Irreversible Effects

The available human data are of little value in helping to ascertain possible effects of repeated exposures. In the study by Ferguson *et al.*,<sup>4</sup> there was no evidence of adverse effects in human subjects exposed to

varying concentrations (25-100 ppm) for varying periods (2-6 hours) daily, 5 days a week, for 6 weeks; however, these exposure levels are below possible exposures in the military setting. Such data as are available are contained in animal studies, and as noted earlier, extrapolation of the animal data to man is complicated by the apparent wide range of susceptibilities among the species. The main question to be answered in this connection is whether repeated exposures in the range of concentrations found to be barely tolerable by highly motivated subjects (say, 400-500 ppm) will produce either reversible or irreversible structural changes in the lower respiratory tract.

Effects associated with prolonged exposures of animals to high levels of ammonia, either in repeated or continuous doses, are displayed in Table 2, together with concentration, time, and dosage (Ct) values. For purposes of comparison, based on the guidelines of the procurement and data on the highest peak ammonia concentrations ever measured in the confined space of armored vehicles, the worst-case dose (Ct) under military operational conditions probably would never exceed 34,400 ppm-hours (410 ppm for 1 hour, 6 times a day, for 14 days).

Thickening of the tracheal mucosa and a reduction in the number of goblet cells were demonstrated after the second week of exposure in pigs exposed continuously to approximately 100 ppm of ammonia (a Ct dose of 33,600 ppm-hours).<sup>9</sup> However, pigs appear to be the most susceptible of the several species and similar effects were not reported in two other pig studies.<sup>10,11</sup> Damaged tracheal mucosae were also demonstrated in rats in response to repeated exposures to approximately 100 ppm of ammonia, 5 hours per day, five days a week, for 12 weeks (Ct = 30,600 ppm)<sup>16</sup>. However, in other studies, no histopathologic changes were found in the lungs of any of several species, including rats, after repeated exposures to 221 ppm, 8 hours per day, 5 days per week, for 6 weeks (Ct = 53,640 ppm-hours), and only nonspecific inflammatory changes were found in the lungs of rats and guinea pigs after repeated exposures to 1100 ppm according to the same schedule (Ct = 264,000 ppm-hours).<sup>7</sup>

Aside from differences in susceptibility among the species used, apparent discrepancies in the findings of the several studies may be due to differences in dosage schedules (i.e., there is some evidence to suggest that intermittent exposures may be less toxic than continuous exposures) or, perhaps more importantly, to differences in endpoint determinations (e.g., an overall review of histopathologic sections of lung and contiguous pulmonary tissues as accomplished by some investigators might not reveal the specific changes in the tracheal mucosa observed by others). None of the studies has addressed the question of whether the observed changes are reversible, disappearing after intervals of no exposure, or if the changes continue to evolve after exposure to produce permanent tissue changes.

There is a small amount of evidence to suggest that damage of the tracheal epithelium is increased by concurrent inhalation of ammonia plus carbon particles (as in smoke) over that produced by inhalation of ammonia alone.<sup>15,16</sup> During the literature review, no evidence was found of enhanced effects of ammonia by interaction with other toxicants or of possible delayed health effects in potentiation of respiratory microbial infection.

TABLE 2

Concentration-Time (Ct) Relationships as Derived From Selected Long, High-Level Exposure Studies

Species	Exposure			Ct (ppm-hours)	Effect <sup>+</sup>
	C	t			
Rats, guinea pigs, rabbits, monkeys, dogs <sup>7</sup>	221 ppm	8 hours per day, 5 days per week, for 6 weeks		53,040	None
	1100 ppm	"		264,000	Nonspecific inflammatory changes in lungs of rats and guinea pigs
	57 ppm	Continuously for 114 days		155,951	None
	181 ppm <sup>*</sup>	Continuously for 90 days		390,960	Nonspecific inflammatory changes in lungs and kidneys
	374 ppm <sup>*</sup>	Continuously for 90 days		807,840	Nonspecific circulatory and degenerative changes in lungs and kidneys
Guinea pigs <sup>8</sup>	650 ppm <sup>*</sup>	Continuously for 65 days		1,014,000	32/51 rats dead by day 25 (390,000 ppm-hours); 50/51 by day 65 (1,014,000 ppm-hours)
	670 ppm	Continuously for 90 days		1,447,200	13/15 rats and 4/15 guinea pigs dead (time unspecified); histopathologic changes in several organ systems in several animals of each species
	170 ppm	6 hours per day, 5 days per week, for 6, 12 and 18 weeks		30,600-91,800	Significant histopathologic changes in several organ systems after 91,800 ppm-hours
Rats <sup>16</sup>	102 ppm	5 hours per day, 5 days per week, for 12 weeks		30,600	6/10 animals, moderately or severely damaged tracheal mucosa
Pigs <sup>9</sup>	100 ppm	Continuously for 6 weeks		100,800	Tracheal damage at end of week 2 (33,600 ppm-hours)
Pigs <sup>10</sup>	50-150 ppm	Continuously for 5 weeks		42,000-126,000	No changes in nasal turbinates or lung

<sup>7</sup>Rats only<sup>+</sup>Histopathology or death

While there may be some slight concern about the possibility of tracheal mucosal damage under conditions of repeated, worst-case exposures, it seems improbable that anticipated exposures, even though repeated, will result in any permanent health effects. Rather, expected effects will be immediate, reversible, in the category of harassing, and significant only to the extent that they may affect combat efficiency to some degree.

#### IV. DISCUSSION

##### A. Exposure Limits

In 1973, the American Conference of Governmental Industrial Hygienists (ACGIH) adopted a threshold limit value (TLV) of 25 ppm of ammonia as a time-weighted average (TWA).<sup>30</sup> This level was selected to protect workers against nose and respiratory irritation and "...to minimize widespread complaints of discomfort among office workers and similar uninjured individual."<sup>\*</sup>

NIOSH (1974)<sup>25</sup> recommended 50 ppm as an occupational standard, expressed as a ceiling not to be exceeded during a workday. The ceiling of 50 ppm was selected because of reports of nasal irritation in some subjects after 10-minute exposures at these levels. In making the recommendation, it was argued that a standard expressed as a TWA was inappropriate because effects were more dependent upon concentration than duration of exposure, and a standard expressed as a TWA would permit fluctuations above levels (50 ppm) that would produce such irritation.

TLVs developed for use in the workplace would appear to have limited application in military situations. Generally speaking, TLVs are designed to protect the health of workers potentially exposed 8 hours a day, 5 days a week, for a working lifetime. Unlike workers, soldiers (whether in training or combat) may experience periods of intense exposure (i.e., exposures greater than the TLV for several minutes to an hour, repeatedly over a period of 24 hours to several days), separated by intervals of no exposure lasting days, weeks or even months.

The TLVs for ammonia were selected to protect workers against nose and throat irritation and to minimize complaints of discomfort. Selected levels are generally less than those affecting the most susceptible individuals, and well below any reported in the literature as being associated with irreversible structural damage. In contrast, it would seem more appropriate that selection of maximum allowable concentrations for military application should be based on considerations of casualty prevention (i.e., either immediate incapacitation or delayed effects) and with a view toward minimizing impairment of operational efficiency.

<sup>\*</sup>The 1979 ACGIH standard is 25 ppm expressed as a TWA; however, a short-term exposure limit of 35 ppm for 15 minutes has now been included in the standard.<sup>31</sup>

## B. Lethality

Most reports of acute ammonia gas exposure have been concerned with clinical descriptions and postmortem findings.<sup>19-24</sup> The main clinical features of such cases are acute inflammation of the upper and lower respiratory tract and eyes. Such deaths as have occurred generally have been attributed to acute pulmonary edema. Surviving patients with residual pulmonary dysfunction usually have had documented acute lower respiratory tract involvement immediately after the acute exposure; however, such residual impairment would appear to be relatively uncommon. Survivors usually have recovered completely within a few days without evident residual effects.

Data on concentrations producing lethal effects are extremely limited. In only one case report has there been any estimate of the concentration of ammonia to which the victim was exposed. Mulder and Van der Zalm<sup>21</sup> reported the death of a worker 6 hours after exposure to an estimated concentration of 10,000 ppm when a tank of ammonium hydroxide overflowed. The duration of the exposure was not stated. Henderson and Haggard<sup>32</sup> state that 5000 to 10,000 ppm is rapidly fatal. Silver and McGrath<sup>18</sup> determined that the LC<sub>50</sub> for mice for an exposure period of 10 minutes and an observation period of 10 days was approximately 10,150 ppm. Practically all deaths occurred during the 10-minute exposure.

Boyd et al.<sup>12</sup> exposed rabbits and cats to average concentrations of 10,360 ppm of ammonia (the approximate LD<sub>50</sub>) for 1 hour in an attempt to elucidate the mechanisms involved in the production of reported clinical effects. Exposed animals showed a threefold increase in respiratory tract fluid and increased iron content of respiratory tract tissues. The latter finding was taken as evidence that congestion of respiratory tract tissues is a prominent feature of acute ammonia gas poisoning; however, the absence of changes in water or chloride content of respiratory tract tissues or serum chloride could not be reconciled with the usually reported clinical finding of pulmonary edema in patients with acute ammonia gas poisoning.

## C. Immediate, Reversible, Irritant Effects

Responses associated with various concentrations cited in Henderson and Haggard<sup>32</sup> are shown in Table 3. These data are quite old and are sometimes at variance with more recent findings. Patty<sup>33</sup> includes the Henderson and Haggard table in his volume, but states that ammonia is detectable by odor at 5 ppm, that 20 ppm is easily noticeable, and that irritation of the mucous membranes is noticeable at 100 ppm. In the various human studies reviewed in this report, wide variations are reported in the threshold levels at which various symptoms of irritation may appear.

In general, the irritant effects of ammonia inhalation are immediate in onset, making their appearance as soon as exposure begins. The severity of effects would appear to be more dependent upon concentration

TABLE 3

Responses Associated with Various Threshold Concentrations  
of Ammonia as Cited in Henderson and Haggard<sup>32</sup>

- The least detectable odor occurs at 53 ppm
- Maximum concentrations allowable for prolonged exposure is 50-100 ppm
- Maximum concentrations allowable for short exposure (0.5 to 1 hour) is 300-500 ppm
- Least amount causing immediate throat irritation is 408 ppm
- Least amount causing eye irritation is 698 ppm
- Least amount causing coughing is 1720 ppm
- 2500 to 6500 ppm, even for short exposures (0.5 hour), is dangerous
- 5000 to 10,000 ppm is rapidly fatal

than length of exposure, though there may be exceptions to the rule (see Verberk<sup>6</sup> below). In fact, as will be discussed later, several reports of both animal and human exposures suggest that concentrations found to be irritating initially may become more tolerable as exposure continues.

Interpretation of the reports of human studies is difficult primarily because of the lack of any reliable way to measure effects that can be evaluated only subjectively. Even excluding differences in individual sensitivity, there must also be differences in the meaning of terms such as irritating to different individuals. At best, the subjective measures employed in such studies should be regarded as only crude indicators of effect.

Among the several studies reviewed, the lowest level at which odor perception was measured was 30 ppm.<sup>5</sup> Five of six subjects found the odor to be easily noticeable to strong at this level. The lowest level at which eye, nose and throat irritation of some degree was noted by at least some subjects was 50 ppm.<sup>3,5</sup> Although various adjectives were used in the several studies to describe the subjective effects, in general the irritation experienced at these levels may be classified as moderate.

The values cited above represent the least amounts producing effects in some people. Of greater interest from a military standpoint are the concentrations that can (or will) be tolerated by most individuals, provided, of course, that irreversible effects do not ensue. Again, there is great variability among the several groups of subjects with respect to concentrations tolerated. In the Verberk study, all subjects tolerated 2-hour exposures to 110 ppm; however, only the so-called experts tolerated the full 2-hour exposure at 140 ppm. At 140 ppm, some of the nonexperts had to leave the room before the end of the first hour of exposure and the remainder before the end of the second hour.<sup>6</sup> This observation is of interest because it suggests, first of all, that effects at this level may not be altogether concentration-dependent, and second, that tolerance may vary depending on the motivation of subjects.

Verberk<sup>6</sup> attributed the greater staying power of the experts to psychological factors, meaning their knowledge from the literature that ammonia concentrations at these levels, while irritating, would not result in any permanent health effects. An equally plausible explanation is that the experts were more highly motivated to stay simply because more is expected of elite people and groups. The idea that subjects in the several studies were differently motivated gains credibility when one considers that, in the studies by Cole *et al.*<sup>2</sup> and Silverman *et al.*<sup>1</sup> subjects tolerated exposures of approximately 200 to 350 ppm for 20 minutes and 500 ppm for 30 minutes, respectively. In the former study, the doses used, according to the authors "...led (only) to a prickling sensation in the nose and slight dryness of the mouth but no material discomfort."<sup>2</sup>



Lacrimation is one manifestation of the irritant effects of ammonia on the eye. Tears, of course, are produced continuously to some degree, and lacrimation becomes noticeable as an effect only when tear production is excessive. The lacrimatory effect of ammonia exposure warrants consideration as a separate effect because of its potential to impair operational efficiency through interference with tasks involving visual discrimination (e.g., reading instruments or maps and gun-sighting).

In the several human studies considered, the lowest concentration at which lacrimation was reported is 134 ppm.<sup>6</sup> Five of 10 subjects experienced lacrimation at this level.<sup>6</sup> Ferguson *et al.*<sup>4</sup> also reported lacrimation during excursions above 150 ppm in human subjects who were being exposed to average concentrations of 25, 50 or 100 ppm, 6 hours a day, 5 days a week, over a period of 6 weeks. Lacrimation occurred in subjects during excursions above 150 ppm and as late as the sixth week of the exposures, even though subjects were considered to have become acclimated at the lower levels. Silverman *et al.*<sup>1</sup> reported lacrimation in 2 of 7 subjects exposed to 500 ppm of ammonia for 30 minutes by means of breathing apparatus that covered the mouth and nose of the subjects, even though there should have been no direct contact of ammonia with the eyes. Verberk's subjects reported subjective symptoms of eye irritation at the nuisance level during exposures to 110 and 140 ppm; however, no mention is made of lacrimatory effects.<sup>3</sup> Cole's subjects experienced only a prickling sensation in the nose and slight dryness of the mouth during 20-minute exposures to 101-335 ppm of ammonia.<sup>2</sup>

The limited data in the several studies considered here do not permit identification of a precise threshold for the lacrimatory effects. Based on data from the study by Industrial Bio-Test Laboratories,<sup>6</sup> one might conclude that the concentration causing lacrimation in 50 percent of those exposed is about 130 ppm. Individual responses will probably vary widely around this level. The data from Silverman's study<sup>1</sup> suggest that simply providing exposed personnel with protective eye goggles may not completely prevent the lacrimatory response.

Lacrimation has also been reported in pigs exposed to concentrations of ammonia in the range of 50-150 ppm.<sup>9,10</sup> This observation is of interest because it suggests a marked species effect. In studies covering a wide range of species and concentrations, lacrimation apparently has not been a prominent feature of exposures except at very high concentrations (dogs and rabbits at 1100 ppm).<sup>7</sup>

#### D. Immediate Respiratory Effects

Verberk<sup>3</sup> could not demonstrate any significant changes in vital capacity, forced expiratory volume in 1 second (FEV<sub>1</sub>) or forced inspiratory volume in 1 second (FIV<sub>1</sub>) in 16 subjects exposed to concentrations of 50-140 ppm of ammonia for periods up to 2 hours.

Ferguson et al.<sup>4</sup> likewise failed to find any significant changes in forced vital capacity or FEV<sub>1</sub> in subjects exposed to average concentrations of 25-100 ppm for 2 to 6 hours daily, 5 days per week, for 6 weeks.<sup>4</sup>

Of greater interest perhaps are the studies by Silverman et al.<sup>1</sup> and Cole et al.<sup>2</sup> in which ventilatory changes (respiration rate, tidal volume and ventilation minute volume) were measured in human subjects before, during and after ammonia exposures.

Silverman et al.<sup>1</sup> exposed 7 subjects to 500 ppm of ammonia for 30 minutes via a face mask. Ventilation minute volume increased 50 to 250 percent over pre-exposure values. These changes were accompanied by increases in both respiratory frequency and tidal volume. In some subjects the increase was immediate; in others the increase did not occur until several minutes after exposure began. The changes were subject to cyclic variations at 4 to 7-minute intervals. The mechanism for the cyclic variations is unclear, but was assumed by the authors to be due to a reduction in blood carbon dioxide from the hyperventilation. Unfortunately, blood carbon dioxide was not measured. The hypothesis that cyclic variations were due to changes in blood carbon dioxide is supported by the observation that respiratory minute volumes fell below pre-exposure levels at the termination of exposure.

Cole et al.,<sup>2</sup> on the other hand, found that during exposure of their subjects to mean concentrations of ammonia in the range of 151-335 ppm for 20 minutes, the ventilation minute volume during submaximal exercise was significantly reduced on an average of 6 percent (range 3.5 to 10 percent in the different studies compared to breathing air) and was independent of dose. The exercise tidal volume increased at a concentration of 151 ppm, but was significantly reduced during exposure to the two higher concentrations (205 and 335 ppm). The reduction in ventilation minute volume at 151 ppm was due to a decrease in respiratory frequency, while at the two higher concentrations, the decreases in minute volume were due to decreases in tidal volume, and occurred despite significant increases in respiratory frequency. The ventilation responses occurred within a few minutes of the onset of exposure and were reversible. The lowest concentration (101 ppm) was without noticeable effect on ventilation minute volume, tidal volume or respiratory frequency (see Figure A- 2).

The differences in the findings of the two studies with respect to changes in ventilation minute volume cannot easily be explained on the basis of dose. The response of subjects exposed to the highest concentration in Cole's study (335 ppm; range 256-490 ppm)<sup>2</sup> should have been similar to that exhibited by Silverman's subjects exposed to 500 ppm.<sup>1</sup> The explanation may lie with differences in the level of physical activity in the two studies.

#### E. Acclimation

Only one study in the literature (Ferguson et al.)<sup>4</sup> addresses possible effects in human subjects of long, high-level exposures.\* The purpose of the study was mainly to document the physiological responses of human subjects exposed to ammonia concentrations normally encountered in industry, including experimental confirmation of anecdotal reports of inurement to the irritating effects of ammonia resulting from repeated workplace exposures. There is experimental evidence for such acclimation in various animal species, including pigs<sup>9,10</sup> and dogs and rabbits.<sup>7</sup>

Unacclimated male and female volunteers were exposed for varying periods (2-6 hours) daily to concentrations of 25, 50 and 100 ppm of ammonia, 5 days per week, over a period of 6 weeks. Subjects exhibited no significant changes in vital signs or pulmonary function tests. Signs of irritation of the eyes, nose and throat, as determined by a physician's examination, were found to diminish over the 6-week period of exposure.

While the study was, no doubt, intended to gather information to support the industry's contention that customary workplace exposures result in no adverse health effects, and while there were deficiencies in the experimental design, the study provides the only available direct experimental evidence of an adaptive response in humans to ammonia's irritant effects. The data suggest that subjects may become acclimated to the effects after only 1 or 2 weeks of intermittent exposure to varying concentrations for varying durations. The data are insufficient to determine precise levels (durations and concentrations) required to prevent or reduce the effects resulting from subsequent challenges to higher levels; however, it would appear (see Figure 3-A) that relatively low-level exposures (50 ppm for 4 hours per day for a week) ameliorate the effects of subsequent exposures to higher concentrations for longer periods (100 ppm for 6 hours per day).

The authors also claim that during preliminary experiments "...continued exposure in the range of 130 to 150 ppm was tolerable to four subjects after less than two hours adaptation at varying lower levels;" and "...that after 30 minutes of acclimation at 100 ppm, a 30-second exposure at 300 ppm was just barely tolerable."<sup>4</sup> The validity of these assertions cannot be assessed, as the evidence is not

\*For purposes of this report, long, high-level exposures are defined as exposures that exceed one hour at any concentration over 25 ppm.

presented. In any case, the adaptive response would appear to be incomplete, as all subjects exposed for the 6-week period lacrimated during excursions above 150 ppm, apparently regardless of their previous exposures. The data are unenlightening as to whether one might expect soldiers to become adapted to the irritant effects of ammonia under anticipated conditions of exposure (i.e., during a highly irregular, intermittent exposure schedule), though this seems a possibility.

The overall impression created by the experience of workers exposed in various industrial settings is that if ammonia concentrations are insufficient to cause acute damage, then one need not expect any damage at all. Any experimental evidence that would suggest otherwise is contained in animal studies. Extrapolation of the animal data to man is especially difficult because of the apparent wide range of susceptibilities to ammonia among the several species.

#### F. General Considerations Underlying the Toxicity of Pulmonary Irritant Gases

Boyd et al.<sup>12</sup> first postulated the existence of a nasobucco-pharyngeal filter that absorbed ammonia gas in the inspired air, protecting the trachea and bronchi from damage. This concept was based on experiments in rabbits that inhaled an approximate LD<sub>50</sub> (5200-12,800 ppm; average 10,360 ppm) for 1 hour, either through a cannula inserted into the trachea or through the nose, mouth and throat. Animals gassed via the nose, mouth and throat had longer survival times after gassing than those gassed via the tracheal cannula. The tracheal and bronchial mucosae were normal in animals gassed via the nose, mouth and throat, whereas there was severe damage of these tissues in animals gassed via the tracheal cannula.

Cralley<sup>13</sup> and Dalhamn and Sjöholm,<sup>14</sup> in a series of elegant experiments using resected sections of rabbit trachea, measured tracheal ciliary beating in response to various ammonia concentrations. Cralley found that 400 ppm of ammonia for 10 minutes, or 500 ppm of ammonia for 5 minutes, caused cessation of ciliary activity without recovery in air.<sup>13</sup> In an elaboration of this work, Dalhamn and Sjöholm,<sup>14</sup> by recording the actual rate of ciliary beating, showed that the critical concentration that only just affected ciliary beating in excised rabbit trachea was around 100 ppm, and that 2000 ppm passed over the nasal cavity was the approximate concentration required to produce 100 ppm in the trachea and some decrement in the rate of tracheal ciliary beating in anesthetized rabbits. At concentrations between 2000 to 3000 ppm, 93 to 96 percent of the gas was adsorbed in the upper tract.<sup>14</sup>

Based on this work, Dalhamn and Sjöholm consider the two major factors determining the toxicity of the pulmonary irritant gases to be the adsorptiveness of the gas in the upper passages and its ability to impair ciliary activity. An irritant gas that impairs ciliary activity at low concentrations and is adsorbed only to a slight degree in the

upper passages will prove to be more toxic than one with the reverse characteristics.<sup>14</sup> Using these criteria, ammonia can be said to be of relatively low toxicity compared with other irritant gases (e.g., sulfur dioxide and nitrogen dioxide).

While the concepts developed above may be useful in understanding the possible genesis of respiratory effects from inhalation of ammonia gas, it would be incorrect to extrapolate the findings with respect to dose in such animal models to man. Clearly, there are differences in the anatomy and adsorptive capacity of the upper tracts, and apparently also differences among species in the sensitivity of respiratory tract cilia to the actions of ammonia. So, for example, while rabbits may be able to tolerate exposure by inhalation to 2000 ppm for 45 minutes without suffering significant effects on the tracheal mucosa, for humans the levels may be considerably lower.

There are very few clues to suggest what these levels may be for man. It can perhaps be assumed that at levels that produce coughing, the adsorptive capacity of the upper tract has been exceeded and significant concentrations have penetrated into the lower tract. Henderson and Haggard state that the least amount causing coughing is 1720 ppm;<sup>32</sup> however, Silverman *et al.*<sup>1</sup> selected 500 ppm for their studies of the respiratory effects of ammonia in human subjects because earlier attempts at 1000 ppm had produced immediate coughing. No coughing was induced at 500 ppm. The authors suggest that at concentrations of 500 ppm the inhaled ammonia was largely absorbed in the upper respiratory passages, and that as exposure continued progressively larger amounts were returned to the expired air.<sup>1</sup> The inference to be drawn is that short exposures (not exceeding 30 minutes) to concentrations somewhere between 500 and 1000 ppm will not produce structural changes in the lower respiratory tract in man. The threshold that induces coughing in man would appear to be above 500 ppm, but probably below 1000 ppm.

The differences between species with respect to the sensitivity of respiratory tract cilia to the actions of ammonia are apparent when one compares data from the studies by Dalhamn and his colleagues of rabbit and rat tracheae. They found, for example, that 500 to 1000 ppm of ammonia produced cessation of ciliary beating after 5 minutes in rabbits;<sup>14</sup> 90 ppm produced cessation of ciliary activity after 10 seconds in comparable studies in rats.<sup>16</sup>

#### G. Effects of Prolonged Exposure with Special Reference to Species Susceptibility

Data on the adverse effects of prolonged exposures to high levels of ammonia, either in repeated or continuous doses, are contained exclusively in animal studies. As previously noted, extrapolation of the animal data to man is difficult because of the apparent wide range of susceptibilities to ammonia among the several species. It has already been suggested that the toxicity of the respiratory irritant gases may

depend on the adsorptive capacity of the upper respiratory tract and the sensitivity of respiratory tract cilia to the ciliostatic actions of the gas. Differences among the species in these two latter respects may also explain apparent differences in susceptibility to pulmonary and systemic effects.

Studies of the effects of prolonged exposure have included various species exposed to ammonia concentrations in the range of 50-1100 ppm, repeatedly or continuously, over a wide range of time periods. Nonspecific tissue changes in a number of organ systems and deaths have been reported.<sup>7-11</sup>

Pigs appear to be among the most susceptible of the several species under consideration. Experiments involving this species were designed to determine the effects of poor air quality, due to the ammonia generated by animal waste and dust, on hog productivity. To this end, exposures were continuous and presumably near the range of concentrations that might be expected in hog barns (25-150 ppm).

In attempting to identify a range of concentrations that would be appropriate to use in their experiments with pigs, Stombaugh et al.<sup>10</sup> first exposed one animal to 280 ppm for 36 hours. The animal exhibited excessive secretions about the nose and mouth and a short, irregular respiratory pattern. After 36 hours of exposure the animal convulsed, and the exposure was terminated. The animal recovered. The authors decided on the basis of this experience that an ammonia concentration of 280 ppm was too high to be used in experiments of 5 weeks long, and selected 150 ppm as an upper limit. Similar experiences have not been reported by investigators using similar concentrations in any other species.

Signs of irritation of the eyes and upper respiratory tract were prominent features of pig exposures to concentrations in the range of 50-150 ppm.<sup>9,10</sup> Stombaugh et al. reported excessive nasal, lacrimal and mouth secretions during exposures to 50, 100 and 150 ppm. The signs were more pronounced at the two highest concentrations and gradually diminished over a period of 1 to 2 weeks, suggesting that the animals were becoming acclimated. Coughing was three times more frequent in animals exposed to 100 and 150 ppm than in control animals.<sup>10</sup> Doig and Willoughby also reported signs of eye irritation in pigs exposed to average concentrations around 100 ppm, which disappeared after the first week, and during subsequent excursions above 150 ppm.<sup>9</sup> Curtis, on the other hand, in experiments at concentrations of 50-75 ppm, noted signs of eye irritation in only one animal exposed to 50 ppm of ammonia.<sup>11</sup>

Aside from the human experiments, in which lacrimation has been reported at concentrations as low as 134 ppm,<sup>6</sup> signs of eye and upper respiratory tract irritation have not been prominent features of exposure of other species, except at concentrations much higher than 100-150 ppm (e.g., mild nasal discharge in 25 percent of rats exposed to 374 ppm and lacrimation in dogs and rabbits exposed to 1100 ppm).<sup>7</sup>

The finding of Doig and Willoughby of significant changes in the tracheal mucosa of pigs exposed to average ammonia concentrations of approximately 100 ppm also deserves special note. The tracheal epithelium of exposed pigs was significantly thicker than in the controls, and there was a significant reduction in the number of goblet cells. The difference was apparent by the end of the second week of exposure and marked by the end of the sixth week.<sup>9</sup> Similar changes were not observed in the other two pig studies;<sup>10,11</sup> however, the concentrations used by Doig and Willoughby were somewhat higher than those in the study by Curtis et al. (i.e., an average concentration of 100 ppm and a range of 52-160 ppm in the former<sup>9</sup> versus 50 or 70 ppm 10 percent in the latter<sup>11</sup>). Stombaugh et al.,<sup>10</sup> while using the same or higher concentrations as Doig and Willoughby, apparently examined only tissues from the nasal turbinates and peripheral lung. The apparent inconsistency in the findings of the two latter studies may be due to differences in the tissues examined.

Tracheal epithelial changes have also been reported by Dalhamn and Reid in rats exposed to ammonia concentrations of approximately 100 ppm and to ammonia in combination with carbon dust.<sup>16</sup> Exposures were 6 hours per day, 5 days a week, for 12 weeks. Tracheal sections of over 50 percent of animals exposed to ammonia alone showed moderate or severe damage. The tracheal epithelium of all animals in the group exposed to the combination of ammonia and carbon dust showed moderate or severe damage. The precise nature of the changes is of some interest for purposes of comparison with those observed by Doig and Willoughby in pigs.<sup>9</sup> The severely damaged epithelium was represented by a single layer of flattened or cuboidal epithelium and almost no cilia remained. Goblet cells were not usually seen. The moderately damaged epithelium was represented by taller cells and the cilia, though present, were clumped. Occasional goblet cells were seen. In normal mucosa, ciliated columnar epithelium was observed and goblet cells were numerous.

Similar histopathologic changes apparently were not observed by Coon et al.<sup>7</sup> in any of several species exposed repeatedly (8 hours per day, 5 days per week, for 6 weeks) to 221 or 1100 ppm of ammonia. Exposed animals included rats, guinea pigs, rabbits, monkeys and dogs. No tissue abnormalities were observed in any of the animals exposed to 221 ppm, and only nonspecific inflammatory changes were observed in the lungs of rats and guinea pigs exposed to 1100 ppm of ammonia. The differences between the findings of Dalhamn and Reid<sup>16</sup> and Coon et al. in their rat studies can perhaps be explained on the basis of differences in exposure schedules and morphometric methods. Both studies employed intermittent exposure schedules; however, while animals in Coon's study were exposed to higher concentrations than in Dalhamn and Reid's study (221 ppm compared with 100 ppm), the duration of the exposures was only 6 weeks in Coon's study compared with 12 weeks in the study by Dalhamn and Reid. Dalhamn and Reid made detailed histopathologic examinations of tracheal mucosa only, whereas Coon examined tissues from several organs, including the lungs. One surmises that the latter examinations, because they were broader in scope, may not have focused specifically on the tracheal

mucosa. In any case, one gains the overall impression that, among the several species, pigs exhibit the greatest susceptibility of all, whether measured in terms of manifestations of acute eye and upper respiratory tract irritation, acute systemic intoxication, or histopathologic changes in the respiratory tract.

Besides pigs, rats and guinea pigs appear to be the next most susceptible of the several species. This conclusion is based on a comparison of findings in studies by Coon *et al.*,<sup>7</sup> (1970), in which the several species were exposed in identical fashion to the same concentrations and for the same durations. Nonspecific inflammatory changes were found in the lungs of only rats and guinea pigs exposed repeatedly for 6 weeks to 1100 ppm of ammonia, and not in the lungs of rabbits, monkeys or dogs. During continuous 90-day exposures to 670 ppm, while all species showed rather extensive nonspecific inflammatory and degenerative changes in the lungs, kidneys, heart and liver, deaths occurred in rats and guinea pigs only (13 of 15 rats and 4 of 15 guinea pigs died).<sup>7</sup>

The precise location of man along this spectrum of species susceptibility cannot be determined from the available data. Man seems to resemble the pig in terms of susceptibility to the irritating effects of ammonia on the eyes, nose, mouth and throat. Both man and pig exhibit signs of irritation of the eyes, nose, mouth and throat, including lacrimation, at concentrations between 100 to 150 ppm.<sup>4,6,9,10</sup> However, if coughing is an index of the extent to which ammonia is adsorbed (or not adsorbed) by the upper tract and, therefore, of the degree to which the trachea and lower airways may be protected, man would appear to be less susceptible than the pig, but more susceptible than the rabbit. Pigs cough at 100-150 ppm;<sup>10</sup> man does not cough at 500 ppm but does cough at 1000 ppm;<sup>1</sup> and rabbits adsorb 93 to 96 percent of inhaled ammonia during exposures to 2000-3000 ppm.<sup>15</sup>

#### H. Concentration-Time Relationships

The several chronic exposure studies, especially that by Coon *et al.*<sup>7</sup> permit making some general observations about concentration-time (Ct) relationships.

There is evidence that the immediate effects of ammonia exposure are primarily concentration-dependent and, except at near-lethal concentrations, probably completely reversible. Moreover, as exposure continues, the acute effects disappear suggesting an adaptive response. Such acclimation has been reported in several species, including man.

In contrast, during prolonged exposure to concentrations that are not acutely lethal, either in repeated or continuous doses, there is some evidence that the duration of exposure may be an important determinant of effects. For example, Weatherby<sup>8</sup> demonstrated significant histopathological changes in guinea pigs during repeated exposures (6 hours per day, 5 days a week) to 170 ppm of ammonia after 18 weeks, but not after 12 weeks or 6 weeks.



While the duration of exposure at any given concentration is evidently a significant determinant of effects, concentration would appear to remain the primary determinant (i.e., given equivalent Ct, the effects of exposure to higher concentrations for shorter periods will be more pronounced than to lower concentrations for longer periods); however, there apparently are other determinants as well (i.e., intermittent exposures may be less toxic than continuous exposures). The above generalizations are illustrated by Coon's findings, where continuous exposures of rats to 650 ppm of ammonia produced marked lethality by the 25th day of exposure (390,000 ppm-hours); however, continuous exposures of rats to 181 ppm for 90 days (also approximately 390,000 ppm-hours) produced only nonspecific inflammatory changes of the lungs and kidneys, as did intermittent exposures (8 hours per day, 5 days per week, for 6 weeks) to even higher concentrations but similar Ct (1100 ppm; Ct = 264,000 ppm-hours).<sup>7</sup>

## V. SUGGESTED FOLLOW-ON WORK

The data in the available literature are insufficient to define precise threshold limits for certain of the immediate irritant effects in man. Examples include:

- The least amount producing lacrimation.
- The least amount found to be tolerable or just barely tolerable by highly motivated subjects.
- The least amount producing coughing.

Except in the first case (i.e., the least amount producing lacrimation), having more precise data than are already available on threshold levels may be of little practical consequence. Threshold levels of ammonia producing lacrimation are well below those that highly motivated subjects would find intolerable. Stated differently, by the time concentrations reach intolerable levels, the ability to perform critical tasks will have become so seriously impaired because of the lacrimatory effect, little purpose will be served by remaining in the contaminated environment. In addition, there would appear to be considerable latitude between intolerable concentrations and those associated with significant health effects. Measures to keep ammonia concentrations below the threshold producing lacrimation (e.g., better ventilation systems in armored vehicles) then would certainly also prevent casualties. Given this rationale, the problem simplifies itself to one of determining the least amounts producing lacrimation among the most sensitive individuals and devising means to maintain concentrations below this threshold. Related questions have to do with the possible attenuation of the lacrimatory effect by the wearing of protective goggles or by acclimation through repeated, short exposures to concentrations below the threshold limit.

More definitive answers to questions about possible reversible or irreversible changes in the respiratory tract produced by repeated, short exposures to high levels of ammonia will require additional research in animals. The apparent need for such studies is generated by reports in the literature of tracheal mucosal damage in pigs exposed continuously, and in rats exposed repeatedly to 100 ppm of ammonia for varying durations.<sup>9,16</sup> Neither of the studies addressed the question of whether the observed changes were reversible, disappearing after intervals of no exposure, or irreversible. There are, of course, major differences between the conditions of exposure found to produce these effects in experimental animals and exposures anticipated in troops. Unfortunately, there are no studies in either animals or man that directly address possible effects of repeated exposures of the sort expected under military operational conditions. While soldiers may receive exposures to concentrations greater than 100 ppm (concentrations as high as 410 ppm have been measured during weapons firing in armored vehicles), the durations of such exposure probably will be far shorter than those used in the animal studies. In addition, the experimental

evidence suggests that pigs and rats may be the most susceptible of the several species used in the studies under consideration. Nevertheless, while it can perhaps be assumed that anticipated worst-case exposures under military operational conditions will probably not produce significant reversible or irreversible damage of the respiratory tract, it seems important to have the assurance of direct experimental evidence to this effect. If exposures at these levels are indeed found to produce effects, then for purposes of establishing exposure limits for military application, it would be necessary to know the concentrations and durations of exposure below which effects are not produced and if the damage produced by exposures above these levels is reversible or irreversible.

Studies directed toward these ends might include pigs as experimental animals (selected because they would appear to be the most susceptible of several candidate species), exposed according to schedules thought to be representative of worst-case military conditions (e.g., six 1-hour exposures in a 24-hour period to concentrations around 400 ppm daily for 1 to 14 days). Endpoint determinations might include rather detailed histopathologic examination of sections of tracheae, bronchi, bronchioles and alveoli of animals sacrificed at intervals during exposures and, if until it is apparent that tissues have been restored to normal or the damage is permanent.

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## Appendix A

### REVIEW OF THE LITERATURE

#### 1. HUMAN EXPOSURE STUDIES (See also Summary of Effects Data, Appendix C)

##### Key References:

- Silverman L, Whittenberger JL, Muller J: Physiological response of man to ammonia in low concentrations. J Ind Hyg Toxicol 31(2):74-78, 1949

##### Review:

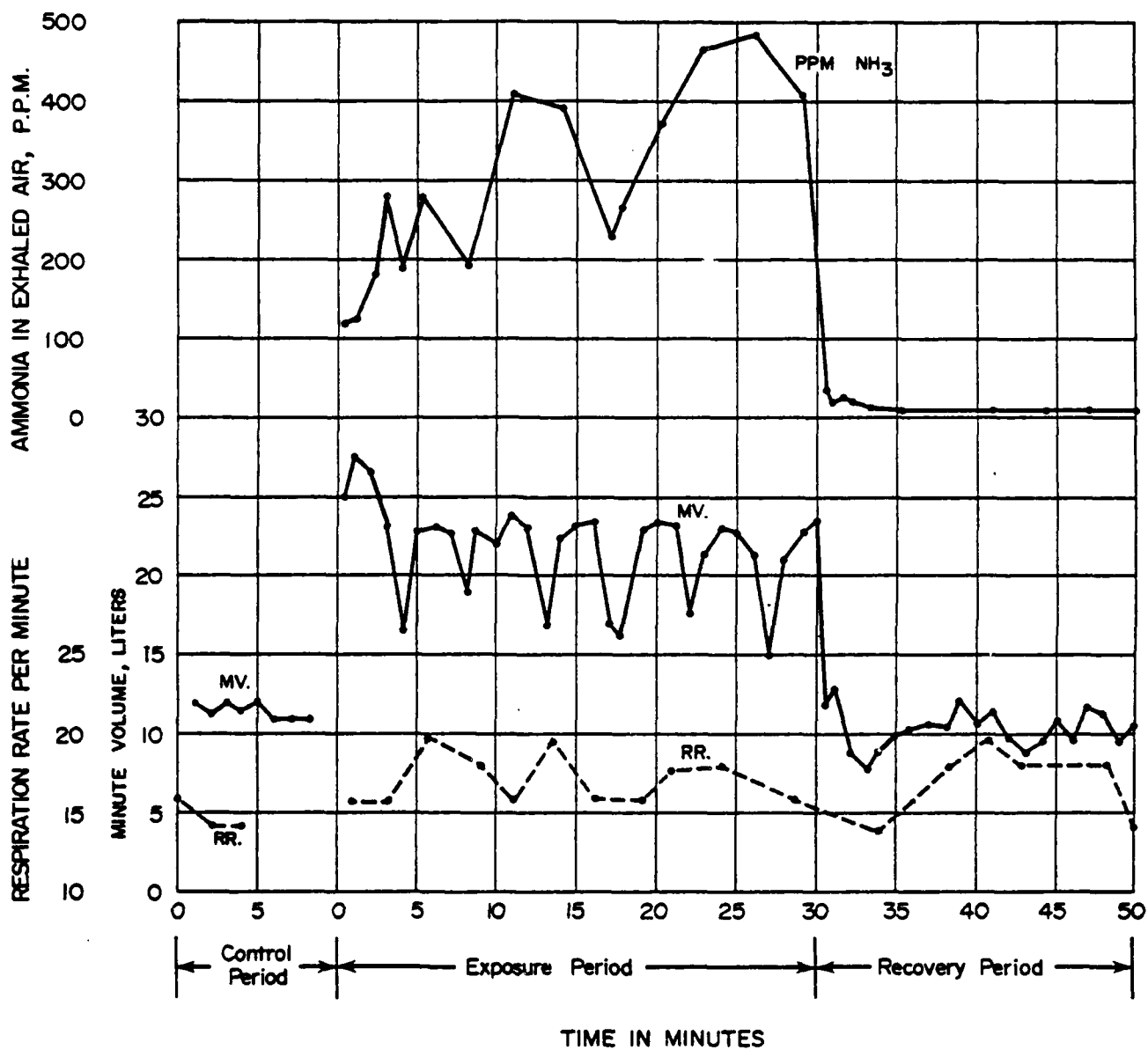
One of the earliest studies of the effects of ammonia on humans was done by Silverman et al. (1949). Adult male subjects were exposed to concentrations of 500 ppm of anhydrous ammonia by means of a breathing apparatus that covered the mouth and nose of the subjects. Initially, two subjects were exposed using this apparatus (five were added later). Blood samples were collected prior to, during, and 30 minutes after exposure for use in determining possible changes in blood urea levels, CO<sub>2</sub> combining power and serum nonprotein nitrogen. Urine samples for the determination of urea and ammonia were collected before and immediately following exposure. Exhaled air samples were collected continuously during and following exposure by means of a sintered absorber placed in the exhaled air stream. Pneumotachograms were taken before exposure and at 5-minute intervals during the exposure and recovery period.

Ammonia concentrations in expired air in a typical experiment are illustrated by the upper curve in Figure A-1, and respiratory rate and minute volume are shown by the lower curves. Expired ammonia levels reached a peak between the 21st and 29th minutes. Silverman states that the cyclic variations in ammonia levels in the expired air correspond roughly to cyclic variation in respiratory minute volume. Equilibrium values and durations of exposure necessary to reach equilibrium are shown for all subjects in Table A-1. At the end of exposure, the ammonia level in expired air fell precipitously, reaching pre-exposure levels in 3 to 8 minutes.

One of the most notable changes observed was the elevation of minute volume over control values, with increases ranging from 50 to 250 percent. An important feature of lung ventilation during the ammonia exposure was the cyclic variation (also illustrated in Figure A-1). The subject represented in Figure A-1 and two others hyperventilated immediately upon exposure to ammonia. In the others, maximum ventilation was not attained for 10 to 30 minutes. At the end of the exposure period, respiratory minute volumes showed a compensatory decrease to below pre-exposure levels, returning to normal within 5 minutes. Respiratory rates consistently increased during ammonia inhalation, but to a smaller degree than ventilation. No coughing was induced, although two subjects experienced lacrimation.

Subjective reactions were obtained from all the subjects, with particular reference to sensations in the upper respiratory tract, eyes and mouth. Reactions of all subjects during the next 24 hours were noted, particularly with regard to any signs of upper respiratory irritation. Two subjects

FIGURE A-1. Ammonia Retention, Minute Volume Changes, and Respiratory Rates for Subject No. 7, Exposure 500 ppm of Ammonia for 30 Minutes\*



\* Adapted from Silverman et al.



TABLE A-1

Physiological Effects of 500 ppm of Anhydrous Ammonia on Seven Subjects \*

No.	Subject	Exposure Time	Recovery Time	Equilibrium Ammonia Concentration in Exhaled Air		Recovery Time for Exhaled Air to Reach Base Level	Mean Minute Volumes*						
							Before Exposure	Maximum During Exposure		Mean During Exposure			During Recovery
				Parts per Million	Time			Amount	Time from Start	Upper	Lower	Cycle frequency	
		(mins.)	(mins)		(mins)	(mins.)	(liters)	(liters)	(mins)	(liters)	(liters)	(mins)	(liters)
1	J.L.W.	30	30	370	22		11.5	23	1	18.5	15		12
2	R.K.	30	30	350	15		17	24	2	23.5	20		13
3	F.J.V.	15	15	350	10	8	6.5	14.5	14	14	8.5	7	7
4	M.W.F.	30	20	370	21	8	8.5	16.5	10	16	12.5	6	15
5	F.E.W.	30	20	400	27	3	9	26	30	24	16	5	7
6	G.J.T.	30	20	370	12	5	9	25.5	12	24	19	4	11
7	B.K.	30	20	480	25	4	11	27.5	1	23.5	17.5	5	11.5

\* Note in subjects 1 and 2, minute volumes were not obtained at 1-minute intervals as in later experiments.

\* Adapted from Silverman et al.

reported 4-hour irritation of the nose and throat. Irritation was likened to a cold with persistent nasal stuffiness. Only two subjects were able to continue nasal breathing throughout the 30-minute exposure. All subjects noted hypoesthesia of the skin around the nose and mouth, which disappeared upon cessation of exposure. Two subjects noted excessive lacrimation even though no ammonia should have come into direct contact with the eyes.

No significant changes were observed in blood or urine nitrogen, blood or urine urea or serum nonprotein nitrogen. Pulse rate and blood pressure were measured in two subjects. One showed no change, while the other showed a very slight elevation of blood pressure and pulse rate.

#### Analysis:

This excellent study leaves little about which to be critical. One may wonder how the investigators managed to gain the apparent willing cooperation of subjects during exposures to such very high concentrations, and whether the observed ventilation effects could possibly have reflected apprehension on the part of the subjects. This seems unlikely inasmuch as maximum ventilatory responses were not attained until 10 to 30 minutes after exposures began in at least some subjects; however, in similar studies, Cole et al. (see below) exposed subjects under conditions of submaximal exercise for the express purpose of reducing the effect of apprehension, which apparently had affected the stability of their results in earlier work. In view of the discrepancies in the findings of Silverman et al. and Cole et al. (in the former study the ventilation minute volume of subjects increased, whereas in the latter study the ventilation minute volume of subjects was reduced), it is unfortunate, by hindsight, that blood carbon dioxide was not measured. Besides being useful in helping to explain the cyclic variations in ventilation minute volume observed by Silverman et al., such data may also represent the key to understanding the discrepancies in observed effects between the two studies.

● Cole TJ, Cotes JE, Johnson GR, Martin HDev, Reed JW, Saunders MJ: Ventilation, cardiac frequency and pattern of breathing during exercise in men exposed to o-chlorobenzylidene malonitrile (CS) and ammonia gas in low concentrations. Q J Exp Physiol 64:341-351, 1977

#### Review:

Cole et al., in a comparison of the effects of ammonia and o-chlorobenzylidene malonitrile (CS) inhalation, studied the ventilation, cardiac frequency and pattern of breathing of healthy young men during exercise. The experiment was divided into three separate studies. In studies 1 and 2, each subject was studied during three consecutive half-day sessions, either mornings or afternoons; the first and third sessions were for control measurements. During the second session of study 2, subjects were exposed to ammonia gas. In study 3, subjects were seen during five consecutive half-day sessions, of which the first, third, and fifth sessions were for control observations. During the second and fourth sessions, subjects were exposed either to ammonia or CS.

Twelve subjects were used in study 1, and 18 each in studies 2 and 3. The subjects all wore full face respirators, which they removed at predetermined times. Nine minutes after removal of the respirator, two periods of exercise using a cycle ergometer were performed. In all but one instance, the exercise was 8 minutes in duration and entailed cycling against increasing loads. In the second period of study 2, the exercise was continued for 11 minutes. Exposures to ammonia gas during four separate periods averaged 101, 151, 205 and 335 ppm. Durations of exposure were around 20 minutes.

During exercise, the subjects breathed through an oro-nasal mask and three-way valve box. Inspiration was from the chamber and expiration was through a mixing bottle into a low resistance gas meter fitted with a photoelectric sensing device. A sample of expired gas was withdrawn continuously from a point distal to the mixing chamber, the CS or ammonia was removed, and the gas was dried and passed through an infrared analyzer for the analysis of carbon dioxide and a paramagnetic analyzer for the analysis of oxygen. The respiratory frequency was obtained either from a thermister in the valve box or from the output of the gas meter. The heart rate was obtained from the electrocardiograph.

The data for each minute of measurement were used to calculate the pulmonary ventilation minute volume ( $V_E$ ), tidal volume ( $V_t$ ), respiratory frequency ( $f_R$ ), cardiac frequency ( $f_C$ ) and the oxygen uptake ( $\dot{V}O_2$ ). These intermediate data were used to obtain by interpolation from appropriate regression relationships the cardiac frequency and ventilation minute volume at the oxygen uptake of  $45 \text{ mmol} \cdot \text{min}^{-1}$  ( $f_{C45}$  and  $V_{E45}$ ) and the tidal volume at the minute volume of  $30 \text{ l} \cdot \text{min}^{-1}$  ( $V_{t30}$ ). Values on control and treatment days with ammonia are shown in Table A-2. Data obtained on treatment days with CS have been omitted.

The subjects reported that the inhalation of ammonia in the doses used led to a prickling sensation in the nose and slight dryness of the mouth but no material discomfort. For mean ammonia concentrations in the range of 151 to 355 ppm, the ventilation minute volume ( $V_{E45}$ ) during exposure was significantly reduced compared with breathing air and was independent of dose. The lowest concentration of 101 ppm was without noticeable effect. The exercise tidal volume ( $V_{t30}$ ) increased at a concentration of 151 ppm, but was significantly reduced at higher concentrations. These results are displayed in Figure A-2.

With ammonia concentrations at the 205- and 335-ppm levels, the reduction in tidal volume ( $V_{t30}$ ) resulted in decreased ventilation minute volume ( $V_{E45}$ ) despite a significant increase in respiratory frequency. At a mean concentration of 151 ppm, however, the reduction in respiratory minute volume ( $V_{E45}$ ) was due to reduced respiratory frequency and occurred despite an increase in tidal volume ( $V_{t30}$ ).

In sum, the authors reported that "in all circumstances, the breathing of polluted air led to a reduction of ventilation minute volume on an average of 6% (range 3.5% to 10% in the different studies). At the higher concentrations of ammonia, such as 205 and 335 ppm, the reduction

TABLE A-2

Mean Gas Concentrations, Exercise Cardiac Frequency, and Ventilation Minute Volume at an Oxygen Uptake of 45 mmol. min<sup>-1</sup> (FC<sub>45</sub> and VE<sub>45</sub>, respectively); also Exercise Tidal Volume and Mean Respiratory frequency at a Ventilation Volume of 30 l. min<sup>-1</sup> (V<sub>t30</sub> and fR<sub>30</sub>, respectively) of Subjects Breathing Ammonia Gas Compared with Breathing Air\*

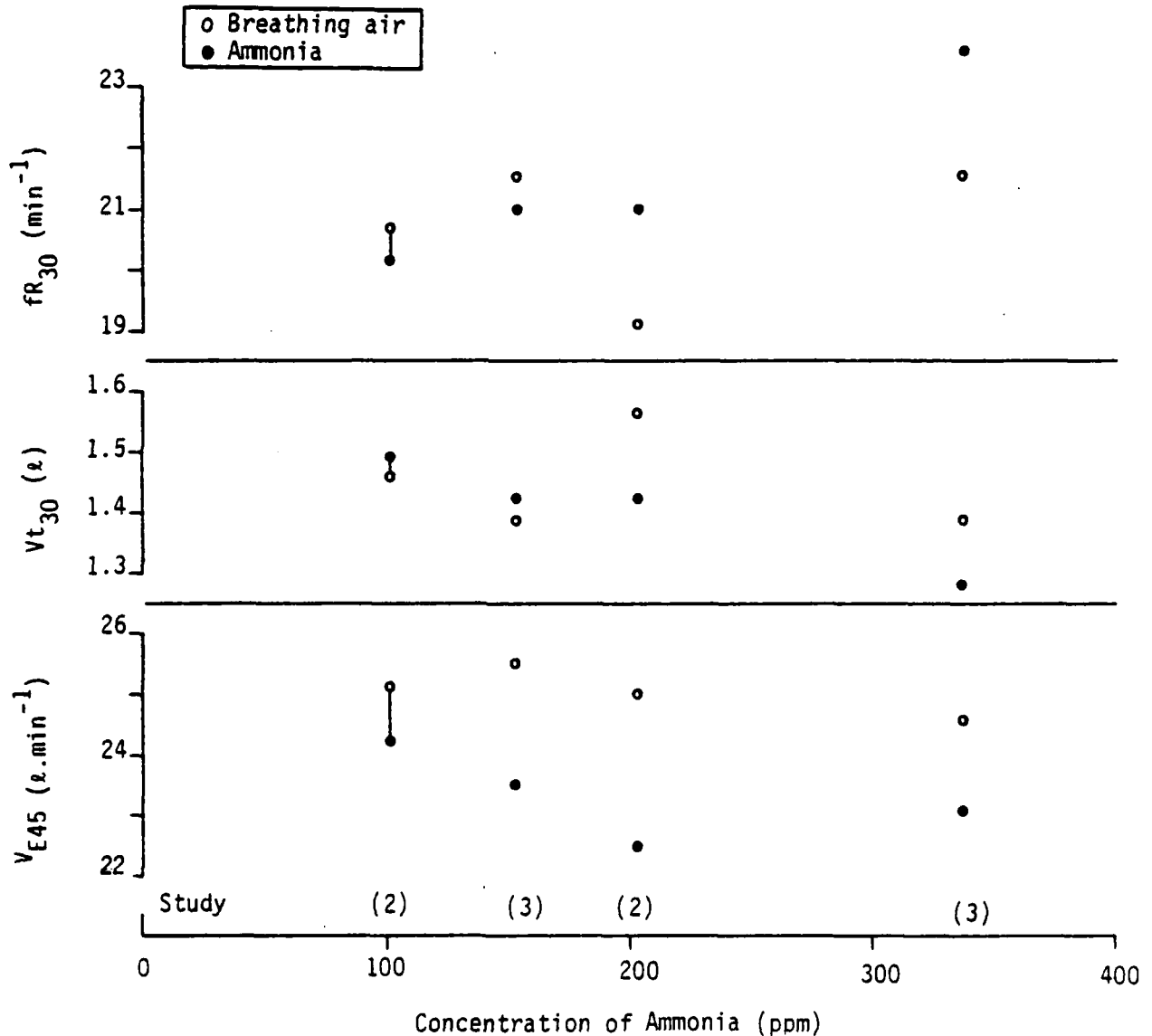
Concentration (ppm)	2nd Study		3rd Study	
	Period 1	Period 2	Period 1	Period 2
	101 (77 - 13)	205 (164 - 225)	151 (103 - 174)	335 (256 - 490)
FC <sub>45</sub> (min <sup>-1</sup> ) (parentheses enclose values adjusted to 20°C)				
Control	144.8 (112)	111.3 (107)	104.3 (104)	99.3 (98)
NH <sub>3</sub>	111.8 (107)	109.1 (102)	107.5 <sup>†</sup> (103)	103.9 <sup>†</sup> (97)
VE <sub>45</sub> (l. min <sup>-1</sup> )				
Control	25.2	25	25.5	24.6
NH <sub>3</sub>	24.3	22.5 <sup>†</sup>	23.5 <sup>†</sup>	23.1 <sup>†</sup>
V <sub>t30</sub> (l)				
Control	1.46	1.57	1.39	1.39
NH <sub>3</sub>	1.49	1.43 <sup>†</sup>	1.43 <sup>†</sup>	1.28 <sup>†</sup>
fR <sub>30</sub> (min <sup>-1</sup> )				
Control	20.5	19.1	21.6	21.6
NH <sub>3</sub>	20.1	21.0 <sup>†</sup>	21.0 <sup>†</sup>	23.4 <sup>†</sup>

\* Adapted from Cole et al.

† Significance of difference from control value p &lt; 0.05.

FIGURE A-2

Effects of Exposure to Different Concentrations of Ammonia Gas on Exercise Respiratory Minute Volume ( $V_{E45}$ ), Tidal Volume ( $V_{t30}$ ) and Respiratory Frequency ( $fR_{30}$ ). (The numbers (2) and (3) designate the two separate studies in which ammonia was used. The vertical lines join pairs of points that do not differ significantly from each other.)\*



\*Adapted from Cole et al.

in respiratory minute volume was due to a reduced tidal volume and occurred despite a small, but in most cases significant, increase in respiratory frequency."

### Analysis

As noted above, one is at a loss to explain the discrepancies in the findings of these two very well-designed studies with respect to ventilation minute volume (Silverman et al. and Cole et al.) except on the basis of the different levels of exercise of the two groups of subjects. Silverman's subjects were at rest; Cole's subject were undergoing submaximal exercise.

- Verberk MM: Effects of ammonia in volunteers. Int Arch Occup Environ Health 39:73-81, 1977

### Review:

Verberk exposed 16 subjects for 2 hours to ammonia in concentrations of 50, 80, 110 and 140 ppm in a study conducted under the auspices of the Committee for Prevention of Disaster by Hazardous Substances in the Netherlands. The purpose of the study was to develop data for use in checking a tentative Public Emergency Limit. The 16 subjects consisted of experts, who were familiar with the effects of ammonia and with laboratory and experimental practices and nonexperts, eight students who had no such familiarity.

Administration of the ammonia gas took place in an exposure chamber where the subjects of each group were exposed to a selected ammonia concentration. Exposures were given to one group one day and to the other group the next. Exposures to the several concentrations were separated by a week. Immediately before and after exposure, vital capacity (VC), the forced expiratory volume in 1 second (FEV<sub>1</sub>) and forced inspiratory volume in 1 second (FIV<sub>1</sub>) were measured by means of a spirometer. During the exposure, each subject recorded subjective feelings according to a checklist of symptoms every 15 minutes. Parameters included smell; taste; irritation of eyes, nose, throat and chest; urge to cough; headache; and general discomfort. The following quantitative scale was used: 0 = no sensation, 1 = just perceptible, 2 = distinctly perceptible, 3 = nuisance, 4 = offensive, 5 = unbearable. Pluses and minuses were used to record sensations judged to be between these levels. A few weeks after exposure, histamine thresholds were determined to ensure that subjects did not have more than normal sensitivity of the bronchial airways to nonspecific irritants.

Results of respiratory function tests showed that no subject demonstrated more than a 10 percent decrease in pre-exposure VC, FEV<sub>1</sub> or FIV<sub>1</sub>. The expert group of subjects seldom rated the exposure as offensive. The nuisance score was given most frequently by members of both groups except at the 140-ppm level, where the nonexpert group felt such severe irritation that they left the exposure chamber prematurely.

These results, according to the author, showed that the subjective responses of the nonexpert group were generally higher than those of the expert group. Since there was a lack of any objective differences between the two groups as determined by respiratory function, and assuming that age does not explain the difference, Verberk hypothesized that psychological factors may have played a role. The students probably had little or no understanding of the pathophysiological significance of the perceived effects. Perceptions of irritation may have been interpreted as a threat to health. The experts, however, expected to endure a fairly high level of irritation, which they realized would do little or no irreversible damage.

#### Analysis:

The applicability of the data obtained from the subjective scoring may be limited. The subjects in each group of four (either experts or nonexperts) were together during the major portion of the exposure and in a position to compare notes, even though they were instructed specifically not to do so. In addition, there is a possibility that certain other kinds of group dynamics were operant. For example, it is not inconceivable that the experts may have tended consistently to underestimate the degree of irritation they were actually experiencing and that they tolerated the 140-ppm exposure because none of them wanted to be the first to leave the chamber. In contrast, the premature departure from the chamber of some of the nonexperts in response to the 140-ppm exposure may have precipitated the simultaneous withdrawal of others. In any case, a better design would have been to expose subjects singly, to have replicated studies of the same subjects at the same concentrations, and to have eliminated possible sequence effects by arranging the exposures randomly rather than stepwise in increasing concentrations.

In spite of these criticisms, the study does provide some useful information on threshold levels at which various irritating effects may be expected to occur.

- Ferguson WS, Koch WC, Webster LB, Gould JR: Human physiological response and adaptation to ammonia. J Occup Med 19(5):319-326, 1977

#### Review:

Ferguson et al. exposed six volunteers (five male and one female; three smokers and three nonsmokers) to concentrations of 25, 50 and 100 ppm of ammonia for varying periods of time each day, 5 days per week, over a period of 6 weeks.\* The subjects were not previously accustomed to working in an ammonia environment.

\*While the study extended over a 6-week period, the first week was devoted to "practice in stabilization of conditions and experimental shakedown." According to the authors, the first week "...resulted in acquisition of very little useful data." The conclusions are actually based on 5 weeks of observations.

Exposures were conducted within an Allied Chemical alkali plant. Preliminary range finding experiments were undertaken to determine areas in the plant where the desired concentrations prevailed and were relatively constant. Suitable industrial areas were identified for the 25- and 50-ppm exposures. For the 100-ppm level, a temporary exposure chamber was constructed, because in areas of the plant where levels of this magnitude occurred, the fugitive nature of the emissions made it impossible to control the exposures at constant levels. Apparently there were difficulties in controlling the levels of exposure in the exposure chamber as well, as excursions up to 210 ppm for several minutes were occasionally measured in the exposure chamber.

The six subjects were paired into three groups, identified as A, B and C, containing one smoker and one nonsmoker each. The exposure schedule for the three groups is shown in Table A-3. The normal daily schedule for the subjects consisted of a pre-exposure physician's examination for eye, nose and throat irritation, an exposure, a midpoint physician's observation, a lunch break, a second exposure, and a physician's observation within 30 minutes after the second exposure ended. The degree of irritation was described by the examining physician as follows:

- Mild - pinkish-red color indicating slight injection.
- Moderate - a definite redness.
- Marked - a beefy redness (a level of irritation not reported during the study).

After training by the physician, paired subjects measured their own pulse, respiration rate and pulmonary function (forced vital capacity and forced expiratory volume in 1 second) before, during and after each period of exposure, while briefly absent from the exposure zone. Pulmonary function was measured using a photometric recording spirometer. Complete medical examinations were accomplished by the physician at the end of each week, including observations for abnormal chest sounds, heart murmur, abdominal organ tenderness (liver, spleen and kidneys), neurological responses (reflexes, balance and coordination), and gain or loss of weight.

The respiration rate, pulse, blood pressure and pulmonary function data, as measured before, during and after each exposure, were subjected to statistical analysis using a variance model called "Fixed Effects, 2-Way Classification," to determine if subjects went through a learning process characteristic of many experiments in which acclimation to a foreign atmosphere is experienced. No statistically significant variance was found over time. A three-factor analysis-of-variance model was used to determine if there were statistically significant variances in vital signs and pulmonary function in relation to exposure levels. The only statistically significant variance found was with the FEV<sub>1</sub>, which increased at higher concentrations.

The main findings of interest have to do with the occurrence of eye, nose and throat irritation as observed by the physician. The finding of some degree of irritation (mild and moderate) during exposure was



TABLE A-3

## Exposure Schedule\*

Group	Week					
	1	2	3	4	5	6
A Concentration (ppm)	25	50	100	25	50	100
Duration (hours)	2	4	6	2	4	6
B Concentration (ppm)	50	50	50	50	50	50
Duration (hours)	6	6	6	6	6	6
C Concentration (ppm)	100	50	25	25	50	100
Duration (hours)	6	4	4	2	4	2

\* Adapted from Ferguson et al.

defined as an incident. Not included are observations of mild irritation found both before and after exposure, which was said not to be unusual in the smokers in the group. The authors divided the number of incidents of irritation observed during a given weekly exposure by the number of observations made per individual to obtain ratios of the number of incidents to the number of observations. These data are shown for each subject in Table A-4. The same data are displayed histographically over time for subjects in Groups A, B and C, in Figures A-3, A-4, and A-5, respectively.

At the conclusion of the study, the subjects reported that after the first week they did not suffer any discomfort as the result of exposures up to 100 ppm, and they were unaware of the irritation noted by the physician. During the study, no complaints of discomfort were made to the physician.

In general, subjects showed less irritation as the study progressed. This was true of Group A, where the sequence of exposures in consecutive weeks was 25, 50, 100, 25, 50 and 100 ppm for varying durations, as well as of Group C, where the sequence of exposures was 100, 50, 25, 25, 50 and 100 ppm for varying durations.

During weeks 2 and 5 when all three groups received exposures to 50 ppm, the ratios of total incidents of irritation to observations were 0.30 in week 2 and 0 in week 5. The difference is statistically significant. The tendency toward decreased irritation is not as apparent in Group B subjects who were exposed to concentrations of 50 ppm for 6 hours per day over the entire study; however, the authors emphasize that the rates of irritation in this group were not statistically significantly different from the rate observed in unexposed controls.

During excursions above 150 ppm, all subjects experienced some lacrimation and a sensation of dryness in the nose and throat. One subject showed moderate irritation of the nose after exposure to 100 ppm for 6 hours, during which time there was an excursion to 200 ppm. This occurred in subject 1A at the beginning of week 2. No other cases of moderate irritation were noted throughout the remainder of the study, not even when subjects were exposed up to 6 hours a day to average concentrations of 103, 106, 110, 127 and 140 ppm, with six excursions to 200 ppm (Groups A and C during the last week).

No abnormalities of the chest, heart, abdominal organs, neurological responses, or significant weight changes were found during weekly medical examinations. There also was no apparent impairment of ability to carry out simulated chemical operator's duties consisting of data logging, computational tasks and walking up and down two flights of stairs every half hour.

TABLE 4-A

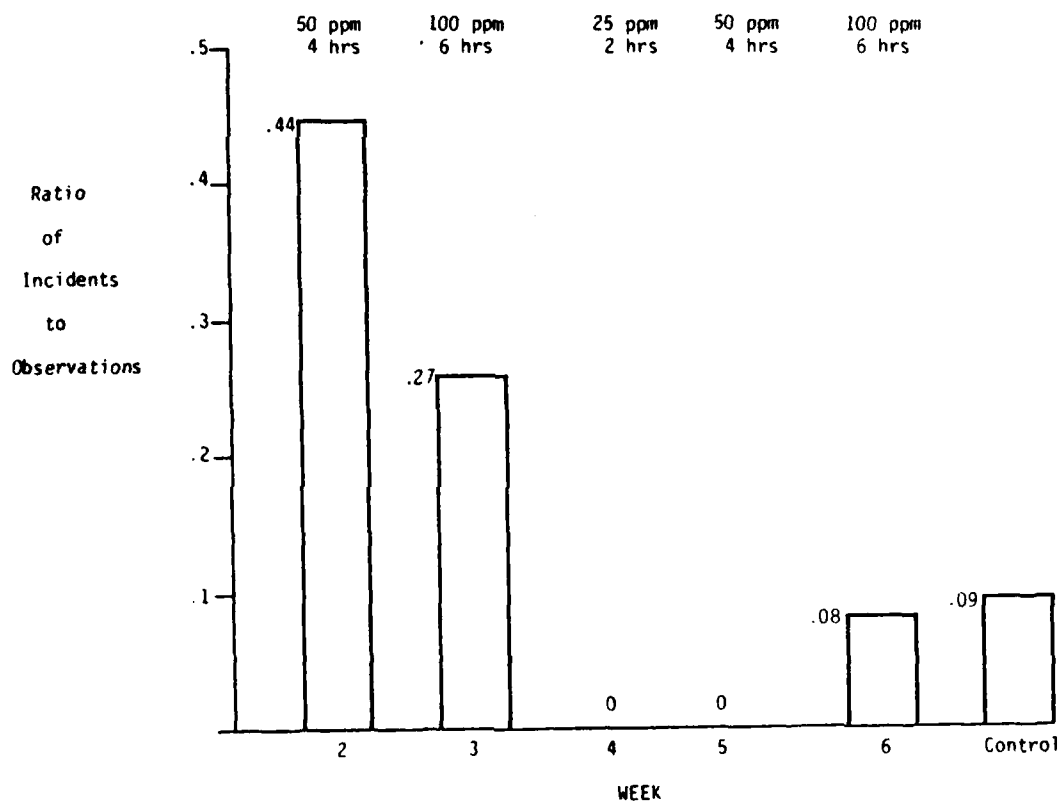
Number of Incidents of Eye, Nose and Throat Irritation (I) Found  
Relation to Number of Observations Made (O), and Ratios of I:O,  
by Individual Subject and Week of Exposure.

Week																
Subject	1	2			3			4			5			6		
	No observations reported	I	O	I/O	I	O	I/O	I	O	I/O	I	O	I/O	I	O	I/O
1A		4	9	.44	4	15	.27	0	15	0	0	9	0	2	12	.07
2A		4	9	.44	4	15	.27	0	15	0	0	9	0	0	12	0
1B		1	9	.11	1	15	.07	0	6	0	0	15	0	2	12	.17
2B		1	9	.11	1	15	.07	2	6	.33	0	15	0	0	13	0
1C		5	9	.55	2	15	.13	0		0	0	15	0	1	15	.07
2C		1	9	.11	0	15	0	0	9	0	0	15	0	0	15	0
Totals		16	54	.30	12	90	.13	2	60	.03	0	78	0	5	78	.06
Controls		0	0	0	0	0	0	1	21	.05	3	12	.25	0	12	0

\*Adapted from Ferguson et al.

FIGURE A-3

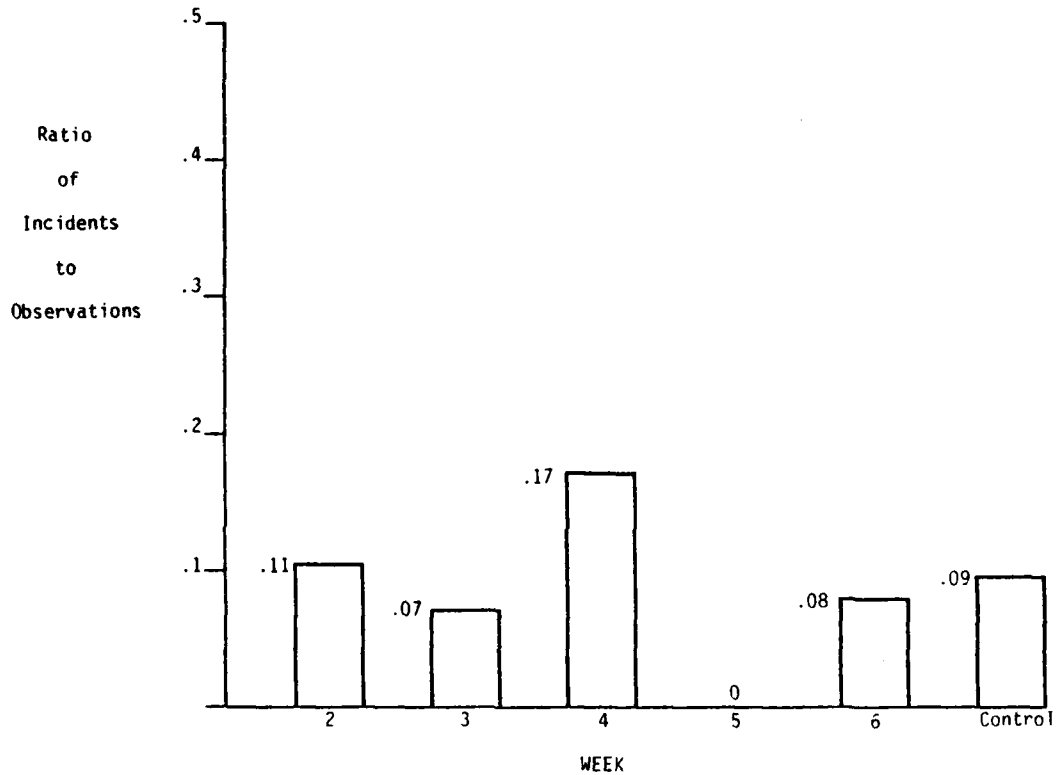
Ratio of Incidents of Eye, Nose or Throat Irritation to Number of Observations Made Over Time in Group A Subjects (Note variable levels of exposure each week)\*



\* Adapted from Ferguson et al.

FIGURE A-4

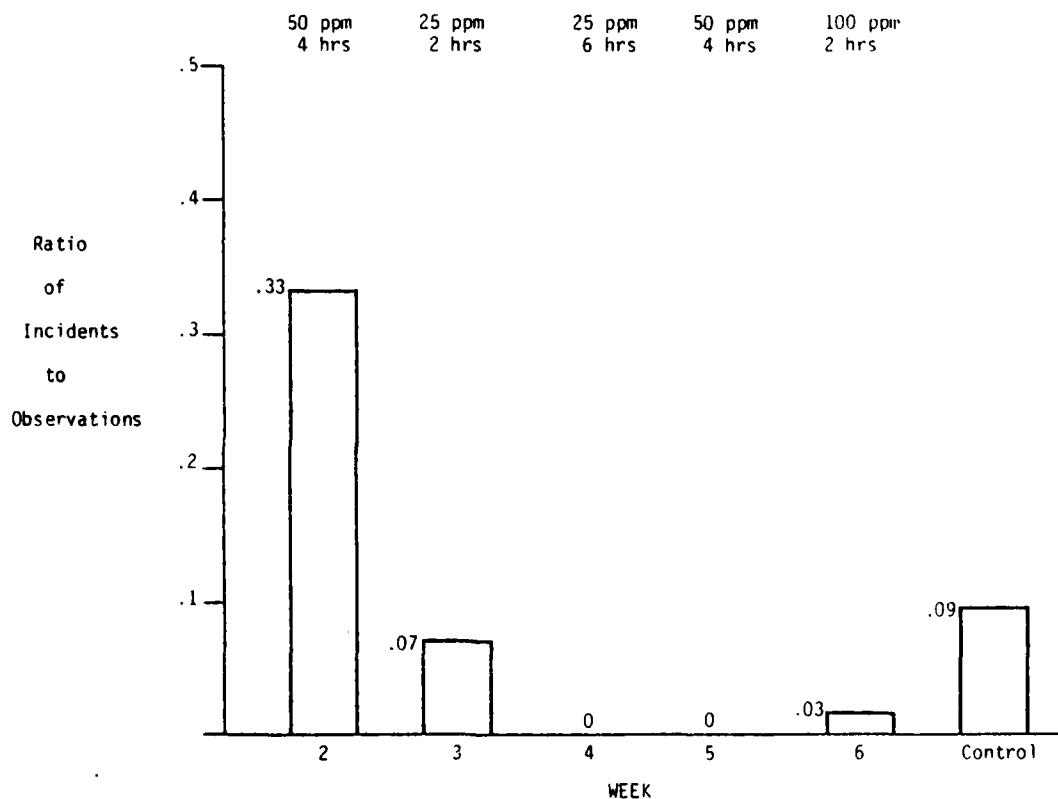
Ratio of Incidents of Eye, Nose or Throat Irritation to Number of Observations Made Over Time in Group B Subjects (All exposures were to 50 ppm for 6 hours per day each week)\*



\* Adapted from Ferguson et al.

FIGURE A-5

Ratio of Incidents of Eye, Nose or Throat Irritation to Number of Observations Made Over Time in Group C Subjects (Note variable levels of exposure each week)\*



\* Adapted from Ferguson et al.

### Analysis:

While the data support the main conclusions of the study, that exposure to ammonia gas in concentrations up to 100 ppm for periods up to 6 hours (with occasional excursions up to 200 ppm) will not result in permanent adverse health effects, and that the irritating effects of the gas on the eyes, nose and throat diminished over time with continued exposure at these levels, there are several major defects in the study design.

Control observations are reported; however, no mention is made of how control subjects were selected or how or when the observations were made. Control observations are not reported until the fourth week of the study, and fewer control observations were made than in exposed subjects. One suspects that the need for a control group was not recognized until well after the study was in progress, and that when the need was recognized, subjects in the various exposure groups became their own controls (i.e., the degree of irritation found at a time when subjects were not exposed represents the control data). The statement "although not shown in the exposure schedule, each group was removed from exposure for one to three days, randomly selected, without the knowledge of the physician, to evaluate the objectivity of the medical examination" lends some credence to this suspicion.

Although the exposure schedule indicates that subjects received exposures for 6 weeks, data from the first week of exposure are not presented. The reason for this is attributed to the requirement for an experimental shakedown. If subjects did receive exposures during week 1, and if previous exposures do indeed mitigate the effects of subsequent exposures, subjects cannot be considered to have been entirely "virgin" at the time observations began to be recorded in week 2, which may somehow have modified the subsequent observed effects.

The physician's observations of the degree of eye, nose and throat irritation are described as objective; yet anyone who has made such observations knows the difficulty of grading something as nonspecific as irritation, and the investigators must have had reason to suspect the physician's objectivity if they found it necessary to have his observations checked by an independent observer. The usual experimental approach to the elimination of such potential observer bias is to ensure that such observations are made at least under single-blind conditions (i.e., the study is designed so that observations of both the experimental subjects, and preferably also suitable controls, are made without knowledge by the observer of the groups to which the subjects are assigned).

While untrained persons certainly can be taught to measure such parameters as pulse, respiration rate and blood pressure and to do pulmonary function tests, one would feel more comfortable about the data

if they had been collected by a fully qualified observer or, as a minimum, if some other means had been devised to ensure the accuracy of the data.

- MacEwen JD, Theodore J, Vernot EH: Human exposure to EEL concentrations of monomethylhydrazine, AMRL-TR-70-102, paper No. 23, in Proceedings 1st Annual Conference on Environmental Toxicology, 9-11 September 1970, Wright-Patterson Air Force Base, Ohio, pp 355-363

#### Review:

MacEwen et al. exposed six subjects to ammonia concentrations of 30 and 50 ppm. The subjects were not made aware of the level to which they were being exposed and exposures were made in random order. Ammonia concentrations were continuously monitored and stabilized in a test chamber. The subjects then inserted their heads through a rubber diaphragm into the chamber and received a 10-minute exposure. The degree of irritation was rated subjectively on a scale of 0-4; odor on a scale of 0-5. The results are shown in Table A-5.

At the 50-ppm exposure level, only moderate irritation was experienced by four of the volunteers, while one reported no irritation. None of the test subjects found 50 ppm to be discomforting or painful. All subjects found the higher exposure to be highly penetrating in terms of odor, while three gave the same rating at the lower level of 30 ppm.

#### Analysis:

The original article could not be located. Data were extracted from Criteria for a Recommended Standard, Occupational Exposure to Ammonia, HEW Publication No. (NIOSH) 74-136, 1974.

- Industrial Bio-Test Laboratories, Inc.: Irritation threshold evaluation study with ammonia. Report to International Institute of Ammonia Refrigeration, IBT-663-03161, March 23, 1973

#### Review:

Industrial Bio-Test Laboratories also evaluated irritation thresholds of ammonia. Ten subjects received exposures to 32, 50, 72 and 134 ppm for 5 minutes. Irritation criteria were annoyance to the eyes, nose, mouth, throat or chest that persisted throughout the exposure period. The investigators felt that the 5-minute exposure time was adequate, since irritation for ammonia depends more upon concentration than upon time of exposure. The frequency of positive findings for the ten subjects was as follows:

- 32 ppm One subject complained of dryness of the nose.
- 50 ppm Two subjects complained of dryness of the nose.
- 72 ppm Three subjects complained of eye irritation; two had nasal irritation; three had throat irritation.
- 134 ppm Five subjects experienced lacrimation and complained of eye irritation; seven had nasal irritation; eight had throat irritation; one complained of chest discomfort.



TABLE A-5

## Subjective Evaluations of Irritation and Odor\*

Subject	Irritation		Odor	
	30 ppm	50 ppm	30 ppm	50 ppm
1	1	2	3	4
2	0	2	4	4
3	0	0	4	4
4	0	1	4	4
5	-	2	-	4
6	1	2	3	4

## Irritant Scale (Nose and Eye)

Degree	Intensity	Description
0	No irritation	Not detectable
1	Faint	Just perceptible, not painful
2	Moderate	Moderate irritation
3	Strong	Discomforting, painful, but may be endured
4	Intolerable	Exceedingly painful, cannot be endured

## Odor Scale

Degree	Intensity	Description
0	No odor	No detectable odor
1	Very faint	Minimum, but positively perceptible odor
2	Faint	Weak odor, readily perceptible
3	Easily noticeable	Moderate intensity
4	Strong	Highly penetrating
5	Very strong	Intense effect

\* From Criteria for a Recommended Standard, Occupational Exposure to Ammonia, HEW Publication No. (NIOSH) 74-136, 1975

Because the only reaction noted at 32 and 50 ppm was a slight dryness of the nose, it was concluded that concentrations of 50 ppm or less did not cause irritation or discomfort.

#### Analysis:

The original article could not be located. Data were extracted from Criteria for a Recommended Standard, Occupational Exposure to Ammonia, HEW Publication No. (NIOSH) 74-136, 1974. It would be useful to know if the subjects responding at lower levels consistently responded at high levels and in which category of response.

#### 2. ANIMAL STUDIES (See also Summary of Effects Data, Appendix C)

##### Key References:

- Coon RA, Jones RA, Jenkins LT Jr., Siegel J: Animal inhalation studies on ammonia, ethylene glycol, formaldehyde, dimethylamine, and ethanol. Toxicol App Pharmacol 16:646-655, 1970

##### Review:

Coon et al. used modified Rochester-type (dynamic) inhalation chambers for chronic exposure studies of ammonia in five species: male and female Sprague-Dawley and Long-Evans-derived rats, male and female Princeton-derived guinea pigs, male New Zealand albino rabbits, male squirrel monkeys and purebred male beagle dogs. Groups of animals were exposed repetitively, 8 hours per day, 5 days per week, for 6 weeks, to 221 and 1100 ppm, or continuously\* for 65-114 consecutive days† to 57, 181, 374, 650 and 670 ppm of ammonia. In three of the experiments (during continuous exposures at 181, 374 and 650 ppm), only rats were used because it appeared from earlier studies that rats were the most susceptible of the several species.

Except in the experiments where only rats were used, exposed animals in each experiment consisted of 15 rats, 15 guinea pigs, 3 rabbits, 3 monkeys and 2 dogs. Control animals were maintained in dynamic chambers without contaminant, but otherwise were handled the same as the experimental animals.

\*"Down time" for feeding and servicing was less than 2.2 percent of total exposure time.

†Exposures were 90 days except during the 650-ppm run in which 50 of the 51 rats were dead by day 65, when the experiment was terminated; the 57-ppm run was for 114 days.

Blood samples were taken before and after the exposure for determination of hemoglobin, hematocrit and leukocyte counts. Animals were routinely checked for visible signs of toxicity, such as behavioral changes, physical appearance, breathing patterns and locomotor activity. At the termination of each experiment, animals were sacrificed and autopsied. Sections of heart, lung, liver, kidney and spleen were taken for histopathologic examination from half the surviving rats and guinea pigs and from all the surviving rabbits, monkeys and dogs.

In animals exposed repeatedly to 221 ppm, there were no deaths or visible signs of toxicity. Hematologic values were normal and there were no gross abnormalities found in the organs or tissues of any animal at autopsy. Histopathologic examinations revealed a focal pneumonitis in one monkey, but no other abnormalities.

In animals exposed repeatedly to 1100 ppm, there also were no deaths; however, mild to moderate dyspnea and lacrimation were noted in the dogs and rabbits during the first week. These signs disappeared during the second week of exposure. At the termination of the experiment, hematologic values were within normal limits and no gross abnormalities were found at autopsy. The lungs of the rats and the guinea pigs showed consistent nonspecific inflammatory changes described as "...more extensive than those seen in control animal"; however, the authors also stated that the examinations "...did not reveal any changes that could be definitely attributed to exposure."

In animals exposed continuously to 57 ppm (114 days) and 181 and 374 ppm (90 days), there were no deaths or pronounced signs of toxicity and no hematologic or gross abnormalities at autopsy. In animals exposed to 57 ppm for 114 days, histopathologic examinations revealed lipid-filled macrophages in the lungs of both dogs, one monkey and one rat. These changes were not felt to be of any clinical significance. In animals exposed to 181 ppm for 90 days (48 rats), histopathologic examinations revealed nonspecific inflammatory changes in the lungs and kidneys of 50 percent of both the experimental and control animals examined.

In animals exposed continuously for 90 days to 650 ppm, 32 of the 51 exposed rats died by day 25 of exposure and 50 died by day 65, when the experiment was terminated. The animals all displayed mild dyspnea and nasal irritation. No histopathologic examinations were done. In the animals exposed continuously for 90 days to 670 ppm, 13 of 15 rats and 4 of 15 guinea pigs died. Marked eye irritation was noted in the dogs and rabbits, manifested by heavy lacrimation in the dogs, and erythema, discharge, and opacity over one-fourth to one-half of the cornea in the rabbits. Hematologic values did not differ significantly from controls. At autopsy, two rabbits showed moderate lung congestion, one dog had a hemorrhagic lesion of the lung, and all animals showed focal or diffuse pneumonitis. Other findings included calcification of renal tubular and

bronchial epithelium, proliferation of renal tubular epithelium, myocardial fibrosis and fatty changes of the liver plate cells in several animals of each species. However, control animals showed similar changes of lesser severity.

#### Analysis:

This work is by far the most extensive single animal toxicity study in the literature, both in terms of the numbers of different species used and the range of exposures. The studies were done expressly for the purpose of gathering data useful in establishing or supporting threshold limit values (TLV) or confined space guidelines (CSG). The latter term is defined as the concentration suggested for confined spaces where humans may be exposed continuously for as long as 90 days. The CSG for ammonia is approximately 25 ppm. The intermittent exposures in this study were 2 and 25 times the 1966 TLV (50 ppm); the continuous exposures were 2, 7, 15, and 25 times the CSG.

While the studies conform meticulously to customary procedures for the conduct of animal toxicity studies, endpoint determinations as reflected in the findings on histopathologic examinations were disappointing and did not support the study's conclusions. For example, the authors concluded that at 25 times the TLV (i.e., at 1100 ppm for 6 hours per day, 5 days a week, for 6 weeks) there was "...increased incidence of and more extensive diffuse interstitial pneumonitis than seen in controls." The conclusion is inconsistent with the finding that "...histopathologic examination did not reveal any changes that could definitely be attributed to the exposures." Similarly, at approximately 24 times the CSG (i.e., at 650 and 670 ppm continuously for 65 and 90 days), while the high mortality in rats and guinea pigs seems clearly attributable to the exposure, the findings on histopathological examinations were again somewhat equivocal in terms of their relationship to the exposure. Similar changes were seen in control animals; they were only of lesser severity.

An important observation in the present context is the apparent greater susceptibility, as reflected by mortality rates, of rats and guinea pigs compared with the rabbits, monkeys and dogs.

• Weatherby JH: Chronic toxicity of ammonia fumes by inhalation. Proc Soc Exp Biol Med 81:300-301, 1952

#### Review:

Weatherby exposed male guinea pigs to approximately 170 ppm of ammonia for 6 hours a day, 5 days a week, for up to 18 weeks. Twelve guinea pigs were used for the experiments and six were maintained as controls. Control animals were denied food and water when experimental animals were in the exposure chamber. At intervals of 6 weeks, four animals from the experimental group and two from the control group were sacrificed. Hearts, lungs, livers, stomachs, small intestines, spleens, kidneys and

suprarenal glands were removed for histopathologic examinations. Ammonia concentrations in the exposure chamber were measured two or three times daily. A concentration of 170 ppm was sought; however, actual levels ranged from 140 to 200 ppm.

Animals sacrificed after 6 and 12 weeks of exposure showed no tissue abnormalities that could be attributed to the exposure; however, animals exposed for 18 weeks showed changes not found in control animals or in exposed animals sacrificed at 6 and 12 weeks. The spleens, livers and kidneys were congested, and the suprarenal glands showed early degenerative changes. The spleens contained greater than normal amounts of hemosiderin, suggesting increased red blood cell destruction. The upper and lower tubules of the kidneys showed fairly marked cloudy swelling with precipitated albumen in the lumens and some casts. The suprarenal glands were not enlarged; however, there was some swelling of cells and degeneration of cytoplasm, as manifested by a loss of the normal granular appearance of cells of the mid and inner zones. The hearts, lungs, stomachs and small intestines showed no consistent changes suggestive of intoxication.

#### Analysis:

While not stated directly, the clear inference drawn in Weatherby's paper is that chronic workplace exposures to ammonia concentrations of around 170 ppm may produce structural damage to several organ systems. Since most of the published literature deals with possible changes in the respiratory tract resulting from ammonia exposure, this work is a welcome addition. It challenges the assumption that if the concentration is not great enough to cause acute effects, no damage should be anticipated. However, there is no discussion of possible mechanisms to account for observed effects and, more importantly, no recognition of possible species differences. The findings, while interesting, do not warrant the inference made.

- Doig PA, Willoughby RA: Response of swine to atmospheric ammonia and organic dust. J Am Vet Med Assoc 159(11):1353-1361, 1971

#### Review:

Doig and Willoughby investigated the effects of ammonia inhalation alone and in combination with organic dusts in specific-pathogen-free-derived Yorkshire Landrace weanling pigs. Four experiments were conducted using six pigs in each exposure group. For each exposure, 6 pigs were also allocated to a second exposure chamber that functioned as a control chamber. Exposures were up to 6 weeks in duration. The nature of the exposures was as follows:

- Ammonia (mean concentration, 106 ppm; range, 52-160 ppm)
- Corn starch dust ( $6.98 \pm 1.03$  mg/cu ft)
- Ammonia (mean concentration, 92.6 ppm; range 29-196 ppm) and corn starch dust ( $6.04 \pm 5.13$  mg/cu ft)

- Ammonia (mean concentrations 106.2 ppm; range 20-203 ppm) and ground corn dust ( $0.30 \pm 0.32$  mg/cu ft). Though not explicitly stated, control animals during this experiment apparently received exposure to ground corn dust.

Daily observations were made of appetite, demeanor, respiratory rates and signs of eye and respiratory irritation. One pig from each group was sacrificed each week. Packed red cell volume, white blood cell count and differential, and total serum lactic dehydrogenase (LDH) were determined on each pig just prior to each experiment and again just before sacrifice. Tracheal swabs for culture for bacteria were taken at autopsy.

The lungs and trachea were removed intact at autopsy and fixed in 10 percent buffered formalin. Sections were taken from the midportion of each lobe of the lung, trachea and a bronchial lymph node for histopathological examination. Specific changes sought, depending on the tissue being examined, were loss of cilia, epithelial thickness, goblet cell numbers and evidence of inflammatory changes.

Temperature and humidity in the exposure and control chambers were comparable during all four experiments. The mean ammonia concentration in the control chamber was 8.0 ppm (generated by animal wastes) and in the exposure chamber during the corn starch dust experiment, 8.9 ppm.

Clinical signs in exposed pigs were mild. Mild photophobia and lacrimation were noted in pigs exposed to 100 ppm of ammonia during the first week of exposure, but these effects disappeared in subsequent weeks, suggesting that the pigs had become acclimated. During excursions over 150 ppm on two occasions, signs of conjunctival irritation were evident in all exposed pigs. Pigs exposed to the combination of ammonia and corn starch dust were more severely affected than those exposed to ammonia alone, in that signs of conjunctival irritation appeared on the first day and persisted for 2 weeks. Signs in the pigs exposed to ammonia plus ground corn dust were similar to those in pigs exposed to ammonia alone. No adverse clinical effects were noted in pigs exposed only to corn starch dust.

No significant differences between exposed and control pigs were found in packed cell volume, white blood cell or differential counts, or LDH. Exposed pigs also did not differ from controls in the type or frequency of bacterial isolations from the trachea. Both the exposed and control pigs showed subpleural hemorrhages, usually in the dorsal diaphragmatic lobes, and lobular atelectasis limited to the apical and cardiac lobes.

Differences were noted on histopathologic examinations. The tracheal epithelium of pigs exposed to 100 ppm of ammonia was significantly thicker than in the controls. The difference was apparent by the end of the second week of exposure, and by the sixth week the tracheal epithelium of the exposed pig was  $41.6 \mu\text{m}$ , compared with a mean thickness of  $18.9 \mu\text{m}$  in control animals sacrificed during week 6. Mean thickness of tracheal epithelium in the six ammonia-exposed pigs, one of which was sacrificed at

the end of each of the 6 weeks of exposure, was  $32.9 \mu\text{m}$  (S.D.  $\pm 7.5 \mu\text{m}$ ), compared with a mean thickness of tracheal epithelium in unexposed (control) animals, also sacrificed weekly over the 6-week period, of  $19.4 \mu\text{m}$  (S.D.  $\pm 2.1 \mu\text{m}$ ). Test of statistical significance apparently were not done. This difference was not observed in pigs exposed only to dust or to ammonia plus dust. A difference was also observed in the number of goblet cells in the tracheal epithelium of pigs exposed to ammonia alone, and to ammonia plus ground corn dust, when compared with controls. Goblet cells were significantly reduced in number by week 2 in pigs exposed only to ammonia (10.3 cells per 500  $\mu\text{m}$  in the exposed pig compared with 22.7 cells per 500  $\mu\text{m}$  in controls). In pigs exposed to ammonia and ground corn dust, the difference was not evident until week 4 (8.5 cells per 500  $\mu\text{m}$  in the exposed animal, compared with 18.3 cells per 500  $\mu\text{m}$  in controls) and thereafter. Changes similar to those observed in the tracheal epithelium of ammonia-exposed pigs were also noted in the turbinate epithelium of pigs exposed for 5 or 6 weeks to ammonia plus either corn starch dust or ground corn dust. The epithelium tended to be thicker than in the control animals, and there was a marked reduction in the number of goblet cells.

No differences between exposed and control animals were found on histopathological examinations of bronchi, bronchioles, bronchial lymph nodes, or alveoli.

#### Analysis:

Results obtained from this series of experiments, while interesting, scarcely lend themselves to quantitative analysis and interpretation. Ammonia concentrations, while they may have averaged around 100 ppm, were subject to wide fluctuations (range 29 to 203 ppm), and there were admitted difficulties in controlling and monitoring the dust concentrations and particle size distribution during the dust experiments. For example, counting and sizing the dust particles revealed high counts of particles in the range of 0.3 to 3.0  $\mu\text{m}$  both in the room air and the air of both chambers. The smaller particles apparently were indigenous to the room and were not due to the addition of the corn dusts. On the other hand, there were considerable differences in the concentration of larger particles (in the range of 3.0 to 10  $\mu\text{m}$ ) during the various dust experiments (e.g., the concentration of large particles during the corn starch dust experiments was 40 times that during the corn dust exposure).

The above discrepancies preclude accurate interpretation of clinical and histopathologic findings during the combined ammonia and dust exposures. One can only speculate that the enhanced irritant effects in pigs exposed to ammonia and corn starch dust were due to solution of ammonia in water absorbed by the large dust particles, which carried the ammonia in concentrated form to the eye, and that the absence of effects on the tracheal epithelium, as compared to pigs exposed to ammonia alone, was due to the trapping of these ammonia-laden particles in the upper tract. This conclusion is supported by the finding of changes in the turbinate epithelium of pigs exposed to the combination of ammonia and

dust similar to those observed in the tracheal epithelium of pigs exposed only to ammonia. The lower concentrations of ground corn dust may have absorbed less ammonia, which would account for the lack of increased signs of eye irritation; since less was absorbed on irrespirable dust particles, more gaseous ammonia could be inhaled, which would account for the decrease in tracheal goblet cells after week 4 among pigs in this group.

In spite of the shortcomings of this work, the finding of significant changes in tracheal mucosa after relatively short exposures (by the second week) to relatively low concentrations of ammonia (52-160 ppm) suggests that pigs may be among the most susceptible of species included in the several studies under consideration.

- Stombaugh DP, Teague HS, Roller WL: Effects of atmospheric ammonia on the pig. J Animal Sci 20:844-847, 1969

#### Review:

Stombaugh et al. exposed four groups of pigs continuously for 5 weeks. Thirty-six Duroc pigs with an average weight of 45.3 kg were used in trial 1 and 36 pigs with an average weight of 62.5 kg were used in trial 2. For each of the trials, pigs were divided into four compartments. Ammonia was metered into three of the four compartments by a pressure-regulated valve into a low pressure chamber, from which it was regulated by needle valves through rotameters into the supply air ducts. The ammonia level in the fourth chamber resulted from waste decomposition. Ammonia concentrations were measured daily. Actual concentrations in trial one averaged 8, 57, 99 and 134 ppm and those in trial 2 averaged 15, 65, 106 and 156 ppm.

The level of 150 ppm was selected as the upper limit of exposure for the experiments, because an earlier exposure of one pig to approximately 280 ppm of ammonia for 36 hours had produced signs of severe irritation, an irregular respiratory pattern, and convulsions. The authors concluded that 280 ppm was too high a concentration to use in experiments of 5 weeks' duration.

Observations were made of feed consumption, growth rate, feed efficiency. Gross microscopic and bacteriological examinations of the bronchi, lungs and turbinates were performed. At the end of the experimental period, three animals were selected from each compartment in trial 1 and 2 animals from each compartment in trial 2 for autopsy. The bronchi, lungs and turbinates were examined grossly, and tissues from the midportion of the ventral turbinate and the cardiac or anterior part of the diaphragmatic part of the lung were examined microscopically. Foci of consolidation noted on gross examination also were examined microscopically. Swabs for bacterial culture were taken from the ethmoid turbinates and from foci of lung consolidation.



Tissue sections from the animals in trial 1 were unsatisfactory for tissue examination because the water used for scalding at slaughter entered the respiratory tract. However, the eight animals in trial 2 showed no gross or microscopic changes attributable to ammonia exposure. Cultures of Corynebacterium and Pasteurella were obtained from the ethmoid turbinates of two animals exposed at the 150-ppm level and from one animal exposed at the 100-ppm level.

Animals exposed to approximately 50, 100 and 150 ppm in both trials exhibited signs of excessive nasal, lacrimal and mouth secretions, which were more pronounced at the two highest concentrations. After 3 to 4 days, the secretory rate in pigs exposed at the 50-ppm level was only slightly greater than that in control animals. After 1 to 2 weeks, the symptoms appeared gradually to diminish in all animals, suggesting that they were becoming acclimated to the exposures. In addition, coughing recorded at the time of weighing was approximately three times more frequent in pigs exposed to 100 and 150 ppm than in pigs exposed to 50 ppm or in control animals.

#### Analysis:

This study is of interest primarily because of the incidental clinical observations made. The occurrence of clinical signs of irritation of the eyes and respiratory tract, including cough, even in animals exposed at the 50-ppm level, suggests that the pig may be among the most susceptible of the several species in the studies under consideration.

In view of the clinical signs, especially the coughing, it is unfortunate that histopathologic examinations of respiratory tract tissues apparently did not include the trachea. Such examinations might also have shown the changes in tracheal mucosal thickness subsequently demonstrated by Doig and Willoughby. As is, the findings are not inconsistent; different tissues apparently were examined.

• Curtis SE, Anderson CR, Simon J, Jensen AH, Day DL, Kelley KW: Effects of aerial ammonia, hydrogen sulfide and swine-house dust on rate of gain and respiratory-tract structure in swine. J Animal Sci 41(3): 735-739, 1975

#### Review:

Curtis et al., using techniques similar to those of Doig and Willoughby, exposed pigs to 50 or 75 ppm of ammonia alone and in combination with various concentrations of commercial swine-finishing house dust (10 or 300 mg/m<sup>3</sup>) or hydrogen sulfide (2 ppm) for 17 to 109 days. Effects on the rate of body weight gain and on the gross and microscopic pathology of the respiratory tract were determined. Concentrations of ammonia and dust were far more carefully controlled (within  $\pm$  10 percent of the desired level) than in the Doig and Willoughby

study. One pig exposed to 50 ppm of ammonia exhibited mild conjunctivitis and blepharitis. The onset and duration of this finding were not stated. None of the animals showed any gross or microscopic abnormalities of the turbinates, trachea or lungs.

#### Analysis:

The finding of Curtis et al. that ammonia alone or in combination with dust failed to alter the respiratory tract structure of pigs agrees with that by Stombaugh et al. for pigs exposed to 100 ppm of ammonia. Curtis' results are at variance with those of Doig and Willoughby for pigs exposed to 100 ppm of ammonia alone or to ammonia plus corn starch dust or ground corn dust. The latter authors reported thickening of the mucosa and decreased populations of goblet cells in the tracheae of pigs exposed to ammonia alone or ammonia plus ground corn dust, and similar changes in the nasal mucosa of animals exposed to ammonia plus corn starch dust.

The discrepancies in the findings of the several groups of workers may be due to differences in the concentrations of ammonia and respirable dust to which animals were exposed and/or to differences in the tissues subjected to microscopic examination. Actual concentrations of ammonia in the Doig and Willoughby study ranged widely around the desired concentration of 100 ppm, and excursions as high as 203 ppm were measured. It is possible that the effects observed on tracheal mucosa by Doig and Willoughby were due to the higher concentrations of exposure. The authors also admitted to difficulties in controlling the dust concentrations around desired levels, and the increased dust levels in exposure chambers (compared with controls) were due almost entirely to particles in the 3- to 10- $\mu$ m range. Particles of this size, of course, would be deposited in the upper respiratory passages where ammonia adsorbed on the dust particles would exert its effects.

Stombaugh et al. stated that they examined tissues from the nasal turbinates and lung. It is impossible to determine if their examinations included only peripheral lung structures or if the tracheal mucosa might also have been examined. If only the former, any changes in the tracheal mucosa obviously would have been overlooked.

- Boyd EM, MacLachlan ML, Perry WF: Experimental ammonia gas poisoning in rabbits and cats. J Ind Hyg Toxicol 26(1):29-34, 1944

Boyd et al. studied the effects of inhalation of ammonia gas on the lungs, respiratory tract fluid and blood of rabbits and cats. The studies were done to attempt to elucidate the mechanisms involved in the production of clinically observed effects of ammonia gas poisoning in the respiratory tract. The animals were exposed in a 400-liter static gassing chamber to approximately 5200 to 12,800 ppm (average 10,360 ppm) of ammonia for 1 hour. These concentrations were selected because they represent an approximate LD<sub>50</sub>.

The authors measured the production of respiratory tract fluid (milliliters per kilogram of body weight in 24 hours) after gassing as compared with controls; the water content of the tissues of the respiratory tract as a reflection of the amount of pulmonary edema; the iron content (micrograms per gram of triturated, dried tissue) of the tissues of the respiratory tract as a reflection of the amount of congestion; the chloride content (milligrams of chloride per gram of triturated, dried tissue) of the tissues of the respiratory tract and serum chloride, also as reflections of the amount of pulmonary edema; blood hemoglobin; and plasma lipids. Rabbits and cats examined 1 or 2 days after gassing showed a two - to threefold increase in the production of respiratory tract fluid. Other findings were:

- No significant increase in the water content of respiratory tract tissues.
- Increased iron content of the tracheal, bronchial and alveolar portions of the respiratory tract, with an especially marked increase in the tracheal portion.
- No effect on chloride content of the lung or serum chloride.
- Increased blood hemoglobin.
- Increased plasma lipids, mostly in the cholesterol fraction.

To facilitate the collection of respiratory tract fluid immediately after gassing, animals were anesthetized and a cannula was ligated into the trachea. However, a large proportion of the animals prepared in this fashion died within 24 hours. To augment the number of animals available for study on the second day after exposure, a different technique was devised. Animals were gassed before being anesthetized and tracheal-cannulated 15 to 20 hours after gassing. Animals in the first group inhaled the ammonia gas directly into the trachea; in the second group, ammonia was inhaled through the nose, mouth and throat before coming in contact with the trachea.

The mean survival time for rabbits gassed after cannulation was 18 hours, compared with a mean survival time of 33 hours in rabbits gassed before cannulation. In animals gassed after cannulation, the tracheae were edematous and the mucosa was necrotic and sloughed off in 80 to 90 percent of the animals. Damage to the bronchial mucosa was less extensive than in the trachea. In animals gassed before cannulation, the tracheal and bronchial mucosa were normal in histologic appearance. There was less difference between the two groups of animals with respect to the appearance of the bronchioles and alveoli. In both groups, approximately 60 percent of the animals had normal-appearing bronchioles; 40 percent had exudate in the lumina and damage to the epithelial lining. The appearance of the alveoli in both groups was identical, with congestion, edema, atelectasis, hemorrhage and emphysema.

These findings led the authors to postulate that the mucosa of the nose, mouth and throat acted as a so-called nasobuccopharyngeal filter, protecting the trachea and bronchi of the experimental animals from damage due to ammonia (but not the bronchioles and alveoli), and that the mucosa of the upper respiratory tract is more resistant to the effects of ammonia than the bronchiolar-alveolar structures.

The authors could not reconcile the absence of changes in the water and chloride content of the tissues of the respiratory tract, and in serum chloride, with the acute pulmonary edema commonly described in ammonia gas poisoning.

#### Analysis:

Under the static gassing conditions used for the exposures, the authors noted that the concentration of ammonia fell rapidly because of absorption by the animal and in water media, and that "...the average concentration was probably not over one half that present at the beginning." No mention is made of the methods used to charge the chamber initially or to determine the ammonia concentration when exposures began. No data are presented on the range of concentrations obtained over the 1-hour exposure period.

While an adequate number of control animals was used and the differences between exposed and control animals are apparent with respect to the output of respiratory tract fluid, iron content of respiratory tract tissues, blood hemoglobin and plasma lipids, no tests of statistical significance were done.

- Cralley LV: The effect of irritant gases upon the rate of ciliary activity. J Ind Hyg Toxicol 24(7):193-198, 1942
- Dalhamn T, Sjöholm J: Studies on SO<sub>2</sub>, NO<sub>2</sub> and NH<sub>3</sub>: Effect on ciliary activity in the rabbit trachea of single in vitro exposure and resorption in rabbit nasal cavity. Acta Physiol Scand 50:287-291, 1963
- Dalhamn T: Effect of ammonia alone and combined with carbon particles on ciliary activity in the rabbit trachea in vivo with studies of the absorption capacity of the nasal cavity. Int J Air Water Pollut 7:531-539, 1963
- Dalhamn T, Reid L: Ciliary activity and histological observations in the trachea after exposure to ammonia and carbon particles, in Davies, CN: Inhaled particles and vapours--II. Elmsford, NY, Pergamon Publishing Company, 1967, pp 299-306
- Dalhamn T: Mucus flow and ciliary activity in the trachea of healthy rats and rats exposed to respiratory irritant gases (SO<sub>2</sub>, NH<sub>3</sub>, HCHO)--VIII. The reaction of the tracheal ciliary activity to single exposure to respiratory irritant gases and studies of the pH. Acta Physiol Scand 36 (suppl 123):93-97, 1956

#### Review:

Cralley studied the effect of ammonia gas on ciliary activity in resected sections of rabbit trachea. The rabbit was used as the experimental animal because of earlier work that demonstrated that the ciliary response of excised human and rabbit mucosa was similar.

The trachea was excised immediately after the rabbit was killed and placed in Ringer's solution at 33° to 34°C until used. A section of trachea about 2 x 4 mm was placed in a tissue chamber, where the exposures to ammonia gas and the observations by light microscopy of ciliary activity were made. The air in the tissue chamber was maintained at 29° C and at a relative humidity above 95 percent by means of a water bath. A lucite rod was used to direct light on the tissue chamber. Excised tissue was discarded when the elapsed time after death of the rabbit was more than 2.5 hours. This was done because earlier work had shown that the cilia became increasingly sensitive as elapsed time after death increased, leading to earlier cessation of activity with exposure. The volume and rate of gas pumped over the tissue were controlled by a calibrated hypodermic syringe (3.8 to 3.9 ml of gas were pumped over the tissue at a rate of 105 strokes per minute), simulating the rate of air flow in the trachea of a living rabbit. A definite amount of irritant gas were measured in the calibrated syringe connected to the tissue chamber and circulated through the closed system until the gas mixture was uniform. The concentration was checked by analysis of the gas mixture.

Control specimens showed no decrease in ciliary activity after exposure to the circulated humid air, free of irritant gas, for more than 15 minutes. Concentrations of ammonia studied were 20, 30, 60, 110, 200, 300 ppm up to 800 ppm, for 3.5 and 10 minutes. -Each experiment was replicated at least three times. The tissues were observed through the microscope to determine the time interval required for complete recovery of ciliary activity in humid air and Ringer's solution after exposure ended.

Exposure to 600 ppm of ammonia for 10 minutes caused cessation of ciliary activity without recovery in Ringer's solution (800 ppm for 5 minutes did not prevent recovery). Exposure to 400 ppm of ammonia for 10 minutes or 500 ppm for 5 minutes caused cessation of ciliary activity without recovery in air. The author emphasized that the zone of exposures (concentration and time) above which complete cessation of ciliary activity occurs in excised tissue is very narrow and that these exposure levels are roughly equivalent to the concentrations reported in the literature as causing immediate throat irritation (400 ppm).

Dalhamn and Sjöholm studied the effects of various gases (ammonia, sulfur dioxide and nitrogen dioxide) on ciliary beating in rabbit trachea in vitro. The excised trachea was opened longitudinally and placed in a moist, thermoregulated tissue chamber. A constant concentration of the gas and air mixture was supplied from a gas mixing apparatus, moistened to 100 percent relative humidity and warmed to 37°C. The gas-air mixture was blown over the long axis of the trachea at a rate of 1 liter per minute. The tracheal mucosa was observed through a light microscope, and the time recorded until ciliary beating stopped. Among the gases studied, widely varying concentrations were required to produce cessation of ciliary beating. For ammonia, 500 to 1000 ppm caused arrest of ciliary activity in 5 minutes. Much lower concentrations of nitrogen dioxide and sulfur dioxide were required to produce arrest.

The authors also reported on the adsorptional capacity of the rabbit nasal cavity for the same gases. For this purpose, the trachea of living, anesthetized rabbits was exposed and cut completely across. A glass tube was inserted into the cranial end of the trachea, while the caudal end was free. The head of the rabbit was placed in a chamber with a constant concentration of gas and the gas-air mixture was sucked through the tracheal cannula for 45 minutes at a rate of 1 liter per minute. Analysis for ammonia in the gas that had passed through the nose was made after 45 minutes. At concentrations between roughly 2000 to 3000 ppm of ammonia, 93 to 96 percent of the gas was adsorbed. Sulfur dioxide also showed a strong tendency of adsorption even at high concentrations (85 to 97 percent after 45 minutes); however, nitrogen dioxide was adsorbed to a much lower degree (31 to 78 percent after 45 minutes), even at the lowest concentration used.

In a second series of experiments in 1963, Dalhamn used the same techniques to determine the concentration of ammonia that would only just affect the rate of ciliary beating in excised rabbit trachea; to determine the concentration that, after passing over mucous membrane of the upper respiratory passages of the rabbit, would be reduced to the same critical level in the trachea; and, finally, to study possible synergistic effects of respirable carbon particles and ammonia on the rate of ciliary beating.

To find the critical range of concentrations at which ciliary beating would only just be affected, high concentrations were used initially and the cilia were observed for reduction in activity. Exposures were 20 minutes in duration. Concentrations were gradually reduced until no qualitative change was discernible. The actual frequency of the beating was not recorded. Concentrations of 460 to 1000 ppm caused complete cessation of ciliary activity, and at 270 to 400 ppm, the ciliary beat either stopped or was greatly reduced. Below 260 ppm, there was no discernible change in ciliary beating unless the beats were actually counted.

To determine the concentration of ammonia that only just affected ciliary activity, rabbit tracheas were then exposed to 75 to 169 ppm of ammonia for 45 minutes. The actual rate of ciliary beating was recorded by cinematography immediately before and after exposure. Pre-exposure ciliary beat rates were between 1111 and 1603 beats per minute. Post-exposure rates averaged 7.5 percent lower at concentrations between 112 and 169 ppm, but there was no difference in mean rates of ciliary beating at concentrations between 75 and 88 ppm. In this way, Dalhamn concluded that the critical level of ammonia that only just affected ciliary activity was around 100 ppm.

In a continuation of the experiment in six rabbits, concentrations of ammonia around 2000 ppm passed over the nasal cavity for a period of 45 minutes were found to produce concentrations of around 100 ppm in the rabbit tracheas. Ten rabbits were then exposed to average concentrations

of around 2200 ppm of ammonia for 45 minutes, and cinematographic recordings of ciliary activity were made at 15, 30 and 45 minutes. A decrement of 17 percent in the mean rate of ciliary beating was observed after 45 minutes of exposure. The difference was statistically significant.

Finally, ten rabbits were also exposed to 2000 ppm of ammonia absorbed on pulverized carbon particles (average concentration, 2.0 mg/m<sup>3</sup>; median particle size, 1  $\mu$ m). The decrement in the mean rate of ciliary beating was 33 percent after 45 minutes of exposure.

Dalhamn and Reid also studied possible synergistic effects of ammonia and pulverized carbon particles on tracheal ciliary activity in rats under conditions of chronic exposure. Four groups of 10 rats each were exposed either to air, carbon particles alone (7 mg/m<sup>3</sup>; median diameter around 1  $\mu$ m), ammonia alone (average concentration of 102 ppm), or to ammonia and carbon particles in combination (119 ppm of ammonia and 3.5 mg of carbon/m<sup>3</sup>). Exposures were 5 hours per day, 5 days per week for 60 days (12 weeks). At the end of the 60-day exposure, the rate of ciliary beating was determined and sections of trachea from the region where the ciliary rate was measured were examined histologically. Sections were examined without knowledge of the group to which the animal belonged.

Neither ammonia nor carbon alone had any effect on the rate of ciliary beating; however, the combination of ammonia and carbon produced a significant reduction in the rate of ciliary beating. Histologic sections of trachea were graded as normal, moderately damaged or severely damaged. In the air controls and the rats exposed to carbon alone, two of the tracheae in each group were considered to be moderately damaged. The remainder were normal. In the rats exposed to ammonia alone, the tracheae were normal in four, moderately damaged in three, and severely damaged in eight. The tracheal epithelium of all animals in the group exposed to the combination of ammonia and carbon dust showed moderate or severe damage.

Earlier (1956) studies by Dalhamn, using preparations of rat trachea exposed to various ammonia concentrations, showed cessation of ciliary motion after 5 seconds of exposure at 90 ppm, after 10 seconds at 45 ppm, after 20 seconds at 20 ppm, after 150 seconds at 6.5 ppm, and after 7 to 8 minutes at 3 ppm. Recovery of ciliary beating occurred in all specimens 10 to 30 seconds after exposure was discontinued. The findings at the two lowest concentrations were not considered to be reliable because the gas sampling method used provided accurate measurements only at concentrations of 5 ppm or more.

#### Analysis:

This rather elegant series of experiments, beginning with the Cralley study and ending with the several investigations of Dalhamn and his colleagues, is of considerable interest because it enlarges on the concept of the nasobuccopharyngeal filter espoused by Boyd et al.

Dalhamn and Sjöholm consider one of the main factors determining the toxicity of pulmonary irritant gases to be their action on the ciliated epithelium of the respiratory tract. The mucus of the respiratory tract (by serving to trap foreign particles in the inspired air) and the cilia (by continuously moving the mucus layer outward along the respiratory passages) constitute an important defense of the respiratory tract. Anything that impairs ciliary activity will result in stasis of the mucus with its trapped particles.

A second major factor determining toxicity, according to Dalhamn and Sjöholm, is the adsorptiveness of the gas in the upper passages. Thus, an irritant gas that impairs ciliary motion at lower concentrations and is adsorbed to a slight degree in the upper passages will likely penetrate more deeply into the respiratory tract and be more toxic than one with the reverse characteristics.

The experimental models used in these studies are regarded by the authors only as qualitative tools by which to show distinct differences between the relevant properties of gases. This is an important point. It would be appropriate, for example, to infer that ammonia, because of its demonstrated high degree of adsorptiveness in the nasal passages of the rabbit and its relatively low degree of ciliostatic action in comparison with other gases, such as  $\text{NO}_2$  and  $\text{SO}_2$ , is less toxic than the other two. However, it would be incorrect to infer on the basis of the data in rabbits that humans can tolerate exposure to about 2000 ppm of ammonia for 45 minutes without suffering changes in the tracheal mucosa. First, the preparations used can scarcely be regarded as physiologic; second, rabbits have a more convoluted upper tract than humans and rarely breathe through the nose; and third, there are apparently major species differences with respect to sensitivity of the cilia to the actions of ammonia (e.g., compare the response in rabbits and rats).

#### Minor References:

- Silver SD, McGrath FP: A comparison of acute toxicities of ethylene imine and ammonia to mice. J Ind Hyg Toxicol 30(1):7-9, 1948

Silver and McGrath reported on the effects of ammonia inhalation in white mice. Groups of 20 mice were exposed to nine concentrations of ammonia, ranging from 8770-12,940 ppm. All exposures were 10 minutes in duration. Exposed mice were observed during exposure and for 10 days thereafter. Median lethal concentrations with ranges of  $\pm .96$  standard errors ( $p = 0.05$ ) were calculated using the mortality at 10 days. Using this method, the  $\text{LC}_{50}$  for ammonia was calculated as  $10,150 \pm 456$  ppm.

The authors reported that the mice exhibited extreme hyperactivity as soon as exposure was initiated. Typical symptoms included tightly closed eyes, gasping, nose-pawing and scratching. Death with convulsions began to occur after the fifth minute, with practically all deaths (93 percent) occurring during the exposure period. Those mice that did not succumb recovered, and in many cases normal behavior was noted within 10 minutes.



### 3. ACCIDENTAL, HIGH-LEVEL HUMAN EXPOSURES

The immediate and persistent effects of accidental exposures of humans to very high concentrations of ammonia have received considerable attention in the literature; however, in only one instance has there been any estimate of the concentration to which the victim was exposed. The various case reports, therefore, are of little value in helping to determine dose-response relationships, and the levels of exposure in such cases were probably far in excess of any exposures likely to be encountered in military combat or training situations. However, the reports provide insights into the nature of the lower respiratory tract pathology that may ensue when the adsorptive capacity of the upper tract for ammonia is overwhelmed. Generally speaking, the effects of such exposures are immediate. Patients suffering delayed, permanent pulmonary effects usually exhibited evidence of acute lower respiratory tract involvement immediately after the exposure. Such effects are surprisingly uncommon. The usual response pattern is complete recovery without apparent residual pulmonary dysfunction. The articles in this category are all regarded as minor references.

- Slot GMJ: Ammonia gas burns--An account of six cases. Lancet 2:1356-1357, 1938

One of the earliest references to high-level ammonia gas exposure was Slot's 1938 report on six cases of acute exposure following rupture of a pipe containing ammonia. Varying degrees of acute inflammation of the respiratory tract were observed, with residual chronic bronchitis evident in two cases. One of the exposed workers died one month after the accident, and the autopsy revealed acute laryngitis, tracheitis, bronchopneumonia and pulmonary edema. The kidneys showed congestion and early hemorrhagic nephritis, which was attributed to toxemia secondary to chemical skin burns caused by the gas. Acute conjunctivitis was present in one patient.

- Caplin M: Ammonia-gas poisoning--Forty-seven cases in a London shelter. Lancet 2:95-96, 1941

A 1941 Study by Caplin reported on the mass exposure to ammonia of 47 persons in a London air raid shelter when a connecting pipe of an ammonia condenser was ruptured. Exposed individuals were divided into groups depending on the extent of respiratory effects, which ranged from mild upper respiratory irritation to severe inflammatory processes of the entire respiratory tract with complications of pulmonary edema and bronchopneumonia. The severity of the effects depended upon the distance of the person from the ammonia source and the duration of exposure. Estimates of exposure concentrations were not given.

Of the 47 individuals involved, 9 had only slight eye and upper respiratory irritation, with complete recovery in several hours. Twenty-seven were judged to be moderately affected, and exhibited more pronounced irritation of the upper respiratory tract, productive cough with blood-stained sputum, and moist rales in the lungs suggesting an

extension of the inflammatory process into the lower respiratory tract. Three of the moderately affected patients developed pulmonary edema within 6 hours and died. Nine others developed bronchopneumonia on the second and third days following exposure and three of these died. The remaining 15 moderately affected persons made an uneventful recovery within 1 week. Eleven patients were judged to be severely affected, with evidence of pulmonary edema, cyanosis, persistent cough with frothy sputum, and intense dyspnea. Seven individuals in the severely affected group died. No observations of the four survivors were reported following their discharge from the hospital.

- Mulder JS, Van der Zalm HO: A fatal case of ammonia poisoning. Tijdsch Geneeskde 45:458-460, 1967

Mulder and Van der Zalm described an acute case of ammonia poisoning that occurred when a tank of ammonium hydroxide overflowed exposing a worker to an estimated concentration of 10,000 ppm. The length of exposure was not stated. The patient immediately began to cough and vomit, and had difficulty breathing. The victim performed "small jobs" for 3 hours before he was seen at a clinic. At that time, his face was red and swollen, he had conjunctivitis, and his mouth and throat were red and raw. His voice was disappearing and he had labored breathing. When his heart stopped, he was revived by cardiac massage and artificial respiration and transferred to a hospital. Six hours after the accident his heart stopped again and he died. Autopsy showed marked inflammation of the respiratory tract. No pulmonary edema was present, but the tracheal epithelium was almost completely denuded.

- Kass I, Zamel N, Dobry CA, Holzer M: Bronchiectasis following ammonia burns of the respiratory tract--A review of two cases. Chest 63:282-285, 1972

Kass et al. reported on two cases of bronchiectasis following 30- to 90-minute exposures to ammonia vapors at an unspecified concentration. Both patients recovered from the acute effects but continued to complain of chronic dyspnea and productive cough for 2 years following exposure. In the patient exposed for 90 minutes, radiologic examination revealed bronchiectasis in practically all segments of the lungs, and pulmonary function tests showed a marked airway obstruction. Hypoxemia was evident and attributed to an uneven distribution between ventilation and perfusion. The 90-minute exposure also resulted in a marked deterioration of vision with bilateral corneal opacities and early cataract changes. The less severe case showed radiologic evidence of bronchiectasis in a few segments of each lobe of the lung.

- Walton M: Industrial ammonia gassing. Br J Ind Med 30:78-86, 1972

Walton reported on four separate incidents of ammonia exposure involving seven men. One exposure resulted in fatality, and the autopsy revealed marked laryngeal edema, acute congestion and edema of the lung, and a loss of bronchial epithelium.

Five survivors who received heavy exposures suffered chemical burns of the nose, mouth and throat, moderate eye irritation, marked dyspnea with cyanosis, cough with blood-stained sputum, and pulmonary congestion. Radiologic examination did not reveal any abnormalities. Pulmonary function tests (forced vital capacity, forced expiratory volume in one second, and gas transfer factor) showed below-average values initially, with gradual improvement during the first 2 years following the accident. Values on one or more of the pulmonary function tests remained below normal in four of the five patients; however, in two cases, abnormal values in the gas transfer factor were attributed to smoking. The sixth patient received a light exposure, and exhibited only mild symptoms of bronchospasm, which disappeared quickly. Pulmonary function tests in the latter case showed a lower than normal gas transfer factor, which also was attributed to cigarette smoking.

- Dupuy R, D'Oblonsky A, Chauveinc L: Toxic gastritis caused by ammonia. Arch Fr Mal Appar Dig 57:819-820, 1968

Dupuy et al. reported on a case of severe gastritis following inhalation of ammonia at an unknown concentration from a ruptured tank truck. The driver immediately exhibited dyspnea and lacrimation. The respiratory symptoms disappeared within 24 hours, but he developed severe, acute gastritis, which was confirmed by gastroscopy. The gastritis improved over the next several months but reappeared later. This apparently is the only published report of damage to the gastrointestinal tract attributed to ammonia exposure.

#### OTHER GENERAL SOURCES

- US Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health: Criteria for a Recommended Standard, Occupational Exposure to Ammonia HEW Publication No. (NIOSH) 74-136, 1974
- Armored Forces Medical Research Laboratory: Project No. 3--Toxic Gases in Armored Vehicles; Final Report on Sub-Project No. 3-13--Determination of the Characteristics and Effects Upon the Crew of Gun Fumes from Firing of the Weapons in the M7 Tank. Fort Knox, Kentucky, April 8, 1943
- Armored Forces Medical Research Laboratory: Project No. 3--Toxic Gases in Armored Vehicles; Final Report on Sub-Project No 3-15-- Determination of the Characteristics and Effects Upon the Crew of Gun Fumes from Firing of the Weapons in the M4A4E1 Tank. Fort Knox, Kentucky, April 29, 1943
- US Army, Aberdeen Proving Ground, Maryland: The relation of toxic gases to equipment design. Contract Number DA36-034-ORD 1638. US Army Human Engineering Laboratory, 1954
- Byerly RG: Unpublished report of data from operational tests of the XM-1 Tank, Material Test Directorate, Aberdeen Proving Ground, Maryland, 1978.

- American Conference of Governmental Industrial Hygienists: Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1973. Cincinnati, ACGIH, 1973.
- American Conference of Governmental Industrial Hygienists: Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1979. Cincinnati, ACGIH, 1979
- Henderson Y, Haggard HW: Noxious Gases and the Principles of Respiration Influencing Their Action. New York, Reinhold Publishing Co., 1943
- Patty FA: Alkaline materials, in Patty FA (ed): Industrial Hygiene and Toxicology, Vol II, 2d ed. New York, Interscience Publishers, 1963

Appendix B

LITERATURE SEARCH STRATEGY

FIGURE B-1

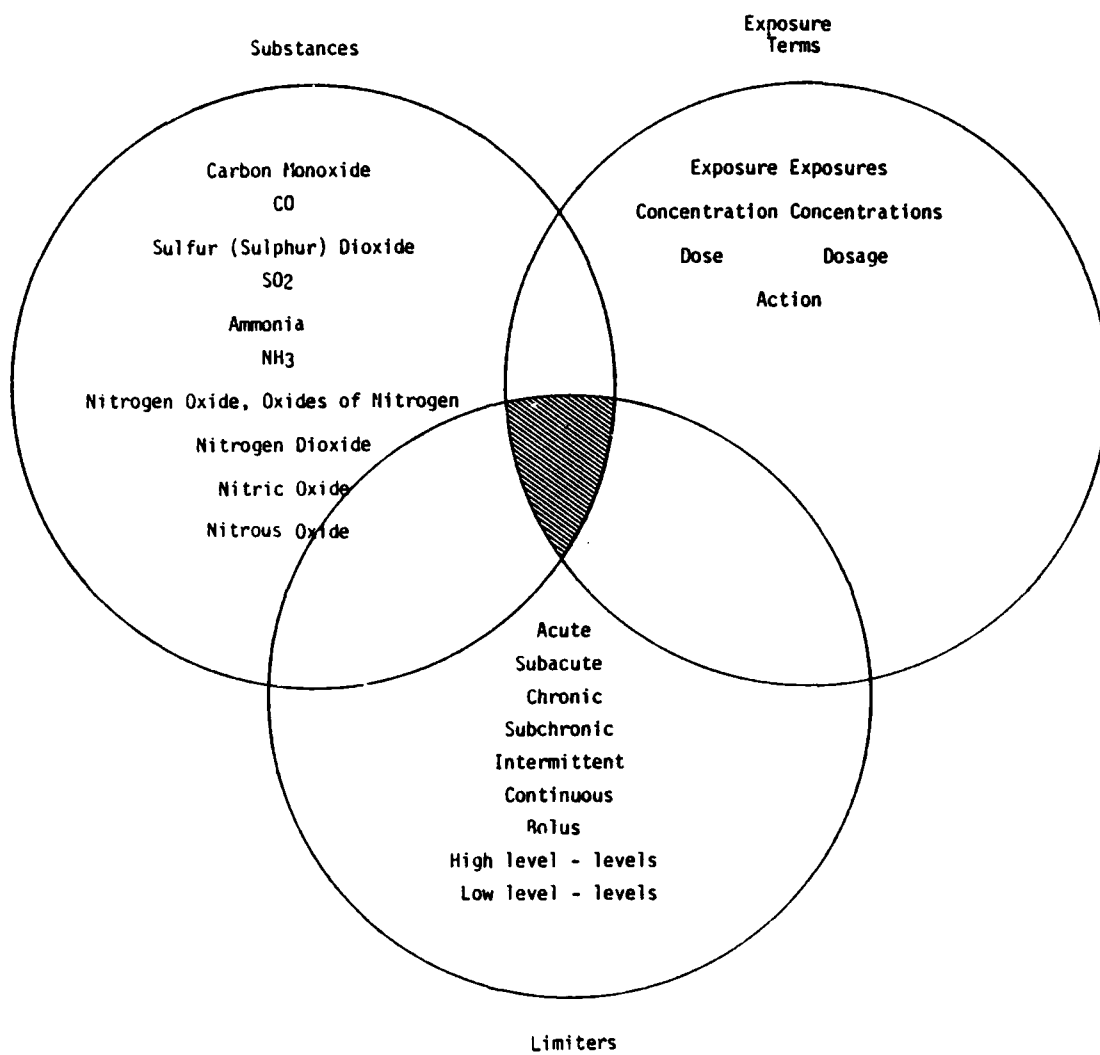
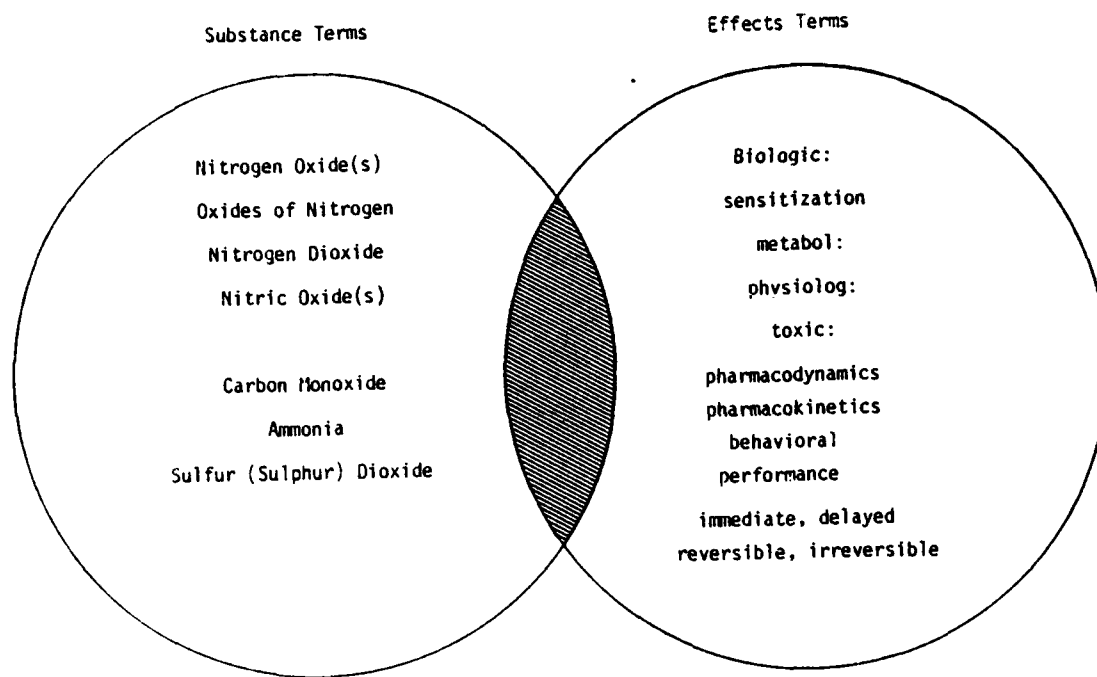


FIGURE B-2



Appendix C

SUMMARY OF EFFECTS DATA AS DESCRIBED IN STUDIES OF SHORT  
AND LONG, HIGH-LEVEL AMMONIA EXPOSURES IN MAN AND ANIMALS



TABLE C-1

Summary of Effects Data as Described in Studies of Short and Long, High-Level Ammonia Exposures in Man and Animals

SPECIES	EXPOSURE		EFFECTS	REFERENCE
	CONCENTRATION	DURATION		
Man (7 subjects)	500 ppm	30 min	<ul style="list-style-type: none"> <li>• Increase in ventilation minute volume of 50-250%, accompanied by cyclic increase in respiratory rate</li> <li>• Irritation of the nose and throat; persistent nasal stuffiness (4 hrs) following exposure.</li> <li>• Lacrimation in two subjects even though no direct contact of <math>\text{NH}_3</math> with the eyes.</li> <li>• No significant change in blood or urine nitrogen, blood or urine urea, or serum nonprotein nitrogen.</li> </ul>	Silverman <u>et al.</u>
Man	101-335 ppm	20 min	<ul style="list-style-type: none"> <li>• Decrease in exercise ventilation minute volume at 151-335 ppm, related either to a decrease in respiratory rate (at 151 ppm) or tidal volume (at 205 and 335 ppm); no significant effects at 101 ppm.</li> </ul>	Cole <u>et al.</u>
Man	50-140 ppm	2 hours	<ul style="list-style-type: none"> <li>• Subjective evaluations of odor, irritation of eyes, nose and throat, and urge to cough on a 5-point scale from 0 = "no sensation," to 5 = "unbearable." Numbers are the numbers of symptoms scored as &gt; 3 ("nuisance") at 0.5-hr mark during a 2-hour exposure. (Each of 8 subjects evaluated 5 parameters.)</li> </ul>	Verberk
8 Experts	50 ppm		2 correct - 40 attempted	
	80 ppm		2 correct - 40 attempted	
	110 ppm		7 correct - 40 attempted	
	140 ppm		11 correct - 40 attempted	
8 Nonexperts	50 ppm		6 correct - 40 attempted	
	80 ppm		12 correct - 40 attempted	
	110 ppm		18 correct - 40 attempted	
	140 ppm		29 correct - 40 attempted	
			<ul style="list-style-type: none"> <li>• 110 ppm tolerable for all subjects.</li> <li>• 140 ppm found intolerable at 1 hour (by 4) and at 2 hrs (by 4) nonexperts.</li> <li>• No significant decrease in vital capacity, <math>\text{FEV}_1</math>, or <math>\text{FIV}_1</math>.</li> </ul>	

TABLE C-1 (cont.)

SPECIES	CONCENTRATION	EXPOSURE	DURATION	EFFECTS	REFERENCE
Man	25, 50, 100 ppm	2-6 hours daily, 5 days per week, for 6 weeks		<ul style="list-style-type: none"> <li>• Mild to moderate irritation as the eyes, nose and throat as determined by physician's examination on 16/54 (30%) of observations on 6 subjects in week 2; 12/90 (13%) in week 3; 2/60 (3%) in week 4; 0/78 in week 5; 5/78 (6%) in week 6.</li> <li>• No apparent effects on pulse, respiration rate, blood pressure, FVC, FEV<sub>1</sub>.</li> </ul>	Ferguson <i>et al.</i>
	150-210 ppm	Brief excursions		<ul style="list-style-type: none"> <li>• Lacrimation and nose and throat dryness in all subjects.</li> </ul>	
Man	30 ppm	10 min		<ul style="list-style-type: none"> <li>• Barely perceptible irritant effects (nose and eye) in 2/6 subjects.</li> <li>• Odor perceived as easily noticeable to strong by 5/6 subjects.</li> </ul>	MacEwen <i>et al.</i>
	50 ppm	10 min		<ul style="list-style-type: none"> <li>• Irritation (nose and eyes) perceived as faint to moderate by 5/6 subjects.</li> <li>• Odor perceived as strong by 6/6 subjects.</li> </ul>	
Man	32 ppm	5 min		<ul style="list-style-type: none"> <li>• Dryness of nose in 1/10 subjects.</li> </ul>	Industrial Bio-Test Laboratories
	50 ppm	5 min		<ul style="list-style-type: none"> <li>• Dryness of nose in 2/10 subjects.</li> </ul>	
	72 ppm	5 min		<ul style="list-style-type: none"> <li>• Eye irritation in 3/10 subjects.</li> <li>• Nasal irritation in 2/10 subjects.</li> <li>• Throat irritation in 3/10 subjects.</li> </ul>	
	134 ppm	5 min		<ul style="list-style-type: none"> <li>• Lacrimation and eye irritation in 5/10 subjects.</li> <li>• Nasal irritation in 7/10 subjects</li> <li>• Throat irritation in 8/10 subjects.</li> <li>• Chest discomfort in 1/10 subjects.</li> </ul>	

TABLE C-1. (cont.)

SPECIES	CONCENTRATION	EXPOSURE DURATION	EFFECTS	REFERENCE
Rats, guinea pigs, rabbits, squirrel monkeys, and beagle dogs	221 ppm	8 hours per day, 5 days per week, for 6 weeks	<ul style="list-style-type: none"> <li>• None.</li> </ul>	Coon <u>et al.</u>
	1100 ppm	8 hours per day, 5 days per week, for 6 weeks	<ul style="list-style-type: none"> <li>• Mild to moderate dyspnea and lacrimation in dogs and rabbits during week 1, which disappeared in week 2.</li> <li>• Nonspecific inflammatory changes in the lungs of rats and guinea pigs.</li> </ul>	
	57 ppm	Continuously for 114 days	<ul style="list-style-type: none"> <li>• No significant tissue pathology.</li> </ul>	
	181 ppm*	Continuously for 90 days	<ul style="list-style-type: none"> <li>• Nonspecific inflammatory changes in the lungs and kidneys of 50% of both experimental and control animals.</li> </ul>	
	374 ppm*	Continuously for 90 days	<ul style="list-style-type: none"> <li>• Mild nasal discharge in 25% of exposed animals.</li> <li>• Nonspecific circulatory and degenerative changes in lungs and kidneys, "difficult to relate specifically to ammonia inhalation."</li> </ul>	
	650 ppm*	Continuously for 90 days	<ul style="list-style-type: none"> <li>• 32/51 rats died by day 25 and 50/51 by day 65.</li> </ul>	
Rats, guinea pigs, rabbits, squirrel monkeys, and beagle dogs	670 ppm	Continuously for 90 days	<ul style="list-style-type: none"> <li>• 13/15 rats died.</li> <li>• 4/15 guinea pigs died.</li> <li>• Marked eye irritation in dogs and rabbits.</li> <li>• Moderate lung congestion in 2 rabbits; hemorrhage in the lung of one dog; all animals showed focal or diffuse pneumonitis; calcification of renal tubular and bronchial epithelium; proliferation of renal tubular epithelium; myocardial fibrosis and fatty changes of liver plate cells in several animals of each species. (Control animals showed similar changes of lesser intensity.)</li> </ul>	Weatherby
	170 ppm	6 hours per day, 5 days per week, for up to 18 weeks	<ul style="list-style-type: none"> <li>• No histopathologic changes in animals exposed 6 or 12 weeks; in animals exposed for 18 weeks, spleens congested; hemosiderosis or spleens; cloudy swelling of upper and lower kidney tubules with precipitated albumen and casts; swelling and degenerative changes of cells in suprarenal glands.</li> </ul>	

\*Rats only

TABLE C-1 (cont.)

SPECIES	CONCENTRATION	EXPOSURE DURATION	EFFECTS	REFERENCES
Swine pigs	100 ppm average (range 20-203 ppm) alone and with organic dust	Continuously for 6 weeks	<ul style="list-style-type: none"> <li>• Mild photophobia and lacrimation during week 1 in pigs exposed to 100 ppm without dust, which disappeared in subsequent weeks.</li> <li>• Conjunctival irritation more severe in pigs exposed to ammonia and corn starch dust, persisting for 2 weeks.</li> <li>• Conjunctival irritation in all animals during excursions above 150 ppm.</li> <li>• Thickened tracheal epithelium and reduced number of goblet cells by week 2 in pigs exposed to 100 ppm without dust; changes more prominent by week 6.</li> </ul>	Doig and Willoughby
Pigs	10, 50, 100 and 150 ppm	Continuously for 5 weeks	<ul style="list-style-type: none"> <li>• Excessive nasal, lacrimal and mouth secretions at 50, 100, and 150 ppm; more pronounced at 100 and 150 ppm, gradually diminishing over 1-2 weeks.</li> <li>• Coughing 3X more frequent at 100 and 150 ppm than at 50 ppm.</li> <li>• No histopathologic changes in nasal turbinates or lung tissue.</li> </ul>	Stombaugh <i>et al.</i>
Pigs	50 ppm alone and with dust (10 mg/m <sup>3</sup> -300 mg/m <sup>3</sup> ) and H <sub>2</sub> S (2 ppm) in several trials; 75 ppm alone in one trial	Continuously for 19-109 days	<ul style="list-style-type: none"> <li>• Mild conjunctivitis and blepharitis in one pig exposed to 50 ppm of NH<sub>3</sub> alone.</li> <li>• No gross or histopathologic abnormalities in turbinates, trachea or lungs in any trial.</li> </ul>	Curtis <i>et al.</i>
Rabbit trachea (in vitro)	20-800 ppm	3-10 min.	<ul style="list-style-type: none"> <li>• Cessation of tracheal ciliary activity without recovery in air at: 400 ppm for 10 minutes, 500 ppm for 5 minutes.</li> </ul>	Cralley
Rabbits trachea (in vitro)	ca. 250-1000 ppm	5-20 min	<ul style="list-style-type: none"> <li>• Cessation of tracheal ciliary activity after 5 minutes at 500-1000 ppm.</li> </ul>	Dahlham
Rabbits (anesthetized, cannulated)	ca. 2000-3000 ppm	45 min	<ul style="list-style-type: none"> <li>• 93-96% of gas resorbed in upper passages.</li> </ul>	Sjoholm

TABLE C-1. (cont.)

SPECIES	EXPOSURE		EFFECTS	REFERENCE
	CONCENTRATION	DURATION		
Rabbit trachea ( <u>in vitro</u> )	75-1000 ppm	20-45 min	<ul style="list-style-type: none"> <li>• Total cessation of tracheal ciliary activity at 460-1000 ppm.</li> <li>• Cessation or reduction of tracheal ciliary activity at 270-400 ppm.</li> <li>• No discernible effect on tracheal ciliary activity at &lt; 260 ppm unless tracheal beats counted.</li> <li>• Least amount that reduces the rate of tracheal ciliary beating, 100 ppm.</li> </ul>	Dahlhamn (1963)
Rabbits (anesthetized, cannulated)	ca. 2200 ppm	45 min	<ul style="list-style-type: none"> <li>• ca. 100 ppm of <math>\text{NH}_3</math> in the trachea and 17% decrement in the rate of tracheal ciliary beating.</li> </ul>	
	2000 ppm with 2.0 mg carbon/ $\text{m}^3$	45 min	<ul style="list-style-type: none"> <li>• 33% decrement in the rate of tracheal ciliary beating.</li> </ul>	
Rats	$\text{NH}_3$ alone (102 ppm)	6 hrs per day, 5 days per week, for 12 weeks	<ul style="list-style-type: none"> <li>• Significant reduction in rate of ciliary beating in combined group.</li> </ul>	Dahlhamn and Reid
	Carbon particles alone (7 mg/ $\text{m}^3$ ) $\text{NH}_3$ (119 ppm) and carbon particles (3.5 mg/ $\text{m}^3$ ) in combination		<ul style="list-style-type: none"> <li>• Histologic sections of trachea: 2/10 controls and 2/9 in carbon-alone group showed moderate damage. 4/10 normal, 3/10 moderately damaged, 3/10 severely damaged in combined group.</li> </ul>	
Rat trachea ( <u>in vitro</u> )	3-90 ppm	10 min	<ul style="list-style-type: none"> <li>• Cessation of tracheal ciliary activity after: 5 sec at 90 ppm, 10 sec at 45 ppm, 20 sec at 20 ppm, 150 sec at 6.5 ppm, 7-8 min at 3 ppm.</li> <li>• Recovery 10-30 sec after exposure ended.</li> </ul>	Dahlhamn (1956)
Mice	8,770-12,940 ppm	10 min	<ul style="list-style-type: none"> <li>• <math>\text{LC}_{50}</math> = 10,150 ppm.</li> </ul>	Silver and McGrath

TABLE C-1. (cont.)

SPECIES	CONCENTRATION	EXPOSURE	DURATION	EFFECTS	REFERENCE
Rabbits and cats	5,200-12,800 ppm		1 hour	<ul style="list-style-type: none"> <li>• Average survival: 18 hr (gassed after cannulation), 33 hr (gassed before cannulation).</li> <li>• 2- to 3-fold increase in production of respiratory tract fluid.</li> <li>• No change in water content of lungs.</li> <li>• Increased iron content of trachea, bronchi, alveolae.</li> <li>• No changes in chloride content of lungs or serum chloride.</li> <li>• Increased blood hemoglobin.</li> <li>• Increased plasma lipids.</li> </ul>	Boyd <u>et al.</u>

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